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China Report

SCIENCE AND TECHNOLOGY

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CHINA REPORT

SCIENCE AND TECHNOLOGY

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PEOPLE'S REPUBLIC OF CHINA

APPLIED SCIENCES

NATIONAL DEVELOPMENTS

SSTC ON SCIENCE, TECHNOLOGY RESTRUCTURING

HK101020 Hong Kong LIAOWANG OVERSEAS EDITION in Chinese No 9, 2 Mar 87 pp 3-5

["New Measures for Promoting the Reform of the Science and Technology Management System--responsible person of the State Scientific and Technological Commission and the Office of the Leading Group for Scientific Work of the State Council answers questions put forward by LIAOWANG reporter"]

[Text] Abstract: In the past year and more, China has taken a significant step in the reform of the science and technology management system. The society at large has recognized technological inventions as commodities. The volume of technological transfers rose from 30 million yuan in 1983 to 2,060 million yuan in 1986, a 68-fold increase. There are about 10,000 organizations combining scientific research with production, an increase of 400 percent over 1984. The state has set up the China Natural Science Fund Committee to provide a stable financial guarantee for the selective support of basic studies, which are in the frontier of science, and some applied studies. The reform of the science and technology management system has stimulated changes in social concepts and a new habit of respecting knowledge and highly trained people has increasingly struck root in the hearts of the people.

However, the divorce of scientific research from production has not been fundamentally changed. Scientific research institutes have not formed a closely interdependent relationship with the national economy. To push forward the reform of the science and technology management system, the State Council recently promulgated the "Decisions on Further Carrying Forward the Reform of the Science and Technology Management System." Its basic contents are: While continuously consolidating, digesting, replenishing, and improving various reform achievements, we should focus on two points: Relaxing policies on scientific research institutes and relaxing policies on scientists and technicians. Relaxing policies on scientific research institutes chiefly means encouraging, by means of preferential policies, most technological development research institutes to go to enterprises or enterprise groups; relaxing policies on scientists and technicians chiefly involves the formulation of appropriate policies and regulations to urge scientists and technicians to go outside their institutes to run small- and medium-sized enterprises under a contract or leasing scheme, to set up various forms of technological development and

services institutions, and to give full play to their talents for the invigoration of the Chinese economy and the development of China's science and technology.

The State Council recently promulgated the "Decisions on Further Carrying Forward the Reform of the Science and Technology Management System." A responsible person of the State Scientific and Technological Commission and the office of the Leading Group for Scientific Work of the State Council answered our report's questions on the promulgation of this regulation.

Question: Will you please talk about the current situation in the reform of China's science and techology management system?

Answer: Since the CPC Central Committee made a decision on reforming the science and technology management system over a year ago, our country has taken a step of decisive significance in the reform of the science and technology management system. The society at large has recognized technological inventions as commodities and technological markets have sprung up in various parts of the country. According to statistics, the volume of technological transfers rose from 30 million yuan in 1983 to 700 million yuan in 1984, to 2,370 million yuan in 1985, and to 2,060 million yuan in 1986. Substantial progress has been made in reforming the science and technology appropriation system. Except for the Chinese Academy of Sciences, a new appropriation system has been implemented throughout the country. In 1986 the nation's expenses in scientific and technological undertakings were cut by an average of 10 percent. A new momentum for lateral association between scientific research and production has emerged. In 1985 [as published] there were about 10,000 organizations combining scientific research with production, an increase of 400 percent over 1984. The vertical distribution of scientific research has been rationally planned and the establishment of the China Natural Science Fund Committee has provided a stable financial guarantee for the selective support of basic studies, which are in the frontier of science, and some applied studies. A special policy has been worked out for the development of scientific and technological undertakings in remote areas. A strategic plan has also been formulated for the development of high technology. The system of employing people for special technical posts is being instituted in a planned way.

What is more significant is that the reform of the science and technology management system has stimulated changes in social concepts. The new habit of respecting knowledge and highly trained people initiated since the 3d Plenary Session of the 11th CPC Central Committee has increasingly struck root in the hearts of the people. Scientific and technological work receive the growing attention of the whole party and society. The social status of intellectuals is gradually rising. Having ease of mind, and being inspired with enthusiasm, scientists and technicians in their millions are plunging into the socialist modernization program with the attitude of being masters of the country. The current spiritual outlook of scientific and technological circles can be regarded as the best since the 1950's.

Question: Why has the State Council decided to push forward the reform of the science and technology management system still further at the moment?

Answer: The reform of the science and technology management system has yielded initial results, but it still faces many problems. The divorce of scientific research from production has not been fundamentally changed. This is demonstrated by the following phenomena: The organizational structure of the departments of science and technology and affiliated organizations has basically remained unchanged and the system of divisions between different departments or regions still exists; the scientific research institutes are still appendages of government departments and they have not formed a closely interdependent relationship with the national economy; due to the lack of effective measures and preferential policies to urge scientific research institutes to combine with enterprises, a number of institutes still take the road of improving themselves and developing their own systems, with the result that not only have few institutes combined with enterprises but the long-standing trend of factory-run scientific research institutes vigorously trying to break away from enterprises still goes on; failure to change the irrational distribution of scientists and technicians has made it impossible for a considerable number of key scientific and technological personnel in big institutes to give full play to their role; fettered by outdated structures and concepts, highly trained people can hardly flow from one place to another, scientists and technicians are censured for taking up concurrent jobs in their spare time, and nongovernmental scientific research institutes do not receive proper attention; and, with the advance of the economic restructuring and unfolding of the reform of the state administrative and management structure, particularly following the reorganization of departments and the transfer of enterprises to a lower administrative level, all departments have been making further efforts to strengthen controls over scientific research institutes.

In view of the above-mentioned circumstances, the State Council has decided to further reform the science and technology management structure. The basic contents of the reform are: While continuously consolidating, digesting, replenishing, and improving various reform achievements, we should focus on two points: First, relaxing policies on scientific research institutes and second, relaxing policies on scientists and technicians.

Question: What is the aim of relaxing policies on scientific research institutes?

Answer: The aim of further relaxing policies on scientific research institutes is to promote multilevel and multiform lateral association between scientific research and production and to expedite the close combination of science and technology with the economy.

Allowing most research institutes of technological development to enter enterprises or enterprise groups to forge a close link between science and

technology and the economy represents an important policy decision in reforming China's science and technology management system; it is also the principal trend of developed countries in developing their economies.

First, this is the practical need for increasing the technological development capabilities of enterprises. With the annual new product rate standing at an average of only 70 percent [as published], our enterprises have been too slow in upgrading products. At this rate, it will take 13 years to upgrade all products. There are many reasons for this. An important one is that our enterprises are weak in technological development. According to the data of a general scientific and technological survey conducted last year, of the 6,000-odd large- and and medium-sized industrial enterprises in our country, only 1,900 had technological development institutions, about 30 percent of which were not quite sound; there were only 100,000 people at or above the engineer level carrying out technological development activities, accounting for only 0.5 percent of the total number of the employees; a total of only 660 million yuan was drawn from the new product development funds last year, accounting for only 0.18 percent of the total sales of products; and one-fifth of enterprises (more than 1,300 in all) had neither funds nor personnel for technological development. If this situation were to continue for a long time, it would be difficult to invigorate the economy. If these enterprises are required to expand their technological development forces, a large amount of money will be needed to set up technological development institutions. Not only will the problem be difficult to solve but there will also be frequent duplication with the existing independent research institutes. What is more difficult is that these enterprises have insufficient technical personnel. In the 6,000-odd large- and medium-sized enterprises there are only 440,000 people at or above the engineer level, some 100,000 of which have been transferred to engage in technological development. Viewed from the current level of enterprise management, this is not a low proportion. Even if we try to transfer more people to do this, there will not be great potential. In the industrial research institutes at or above the prefectural and city levels, there are over 100,000 people at or above the engineer level. If half of them go to enterprises, they will constitute a very strong force. Therefore, a relatively practical method is to encourage the enterprises to form close associations with the existing independent research institutes or to absorb them into the enterprises.

On the other hand, viewed from the present conditions in the scientific research institutes, it is also necessary to do so. Over the years, with the vigorous support of the party and state, the various departments of the State Council and the Chinese Academy of Sciences have set up 1,005 independent research and development institutes (of which, 883 are subordinated to the various departments of the State Council and 122 to the Chinese Academy of Sciences). Although they account for only 20 percent of the total number of research and development institutes (4,951 in all) subordinated to government departments at and above the county levels in the country, the scientists and engineers at their disposal account for

65.5 percent of the total. They are also well-equipped with instruments for scientific research. For this reason, they are a key, decisive force in China's scientific and technological ranks. These institutes have made major contributions in solving major scientific and technological problems for the development of the national economy and the building of national defense, particularly in surmounting highly sophisticated technology. It should be noted, however, that there is still a considerable gap between their contributions and their enormous potentials, that they lack the ability to develop on their own and the vitality to voluntarily serve economic development, and that they are still unable to adapt themselves to the development of the socialist commodity economy and the needs of the new economic structure. Therefore, invigorating these scientific research institutes constitutes an important task related to the overall situation of the reform of China's scientific research structure and also the focus of the work of reforming the science and technology management system at the next step.

With their operating funds reduced year by year, most state-owned industrial research institutes are inferior to large- and medium-sized enterprises in instruments and other equipment. Moreover, when importing advanced foreign technologies, some large- and medium-sized enterprises seldom let them join. All these have resulted in many research institutes lagging behind large- and medium-sized enterprises in terms of technology. If these research institutes, especially the product research institutes, continue to dissociate from enterprises, it will be difficult to run them well.

Over the past few years, the majority of research institutes have energetically implemented the principle of catering to the needs of economic construction in scientific and technological work and thus forged a much closer relationship with enterprises. But this is not enough. There are as many as 10,000 organizations of various types linking scientific research with production but their output value still accounts for a very small proportion in the total value of industrial production. The output value of 450 such organizations in Beijing accounted for only 0.3 percent of the city's total industrial output value in 1985. Only a few large research institutes, such as Pangang Research Institute and Changchun Automobile Research Institute, have established links with enterprises. A considerable number of them are still taking the road of developing on their own and forming their own systems and many factory-run research institutes are still vigorously trying to break away from enterprises.

For this reason, there is an urgent need to work out a policy to guide the enterprises and research institutes to voluntarily form closer associations and forge close cooperation. The principal contents of this policy are as follows:

--Large- and medium-sized industrial enterprises and enterprise groups are explicitly required to have functioning and reliable institutions for technological development. They may transfer personnel to strengthen what

they already have or form close associations with independent institutes. If there are independent institutes of a similar nature, we should try as much as possible to absorb them into the enterprises rather than set up new ones. In the future, we should chiefly rely on a large number of technological development institutions in the enterprises to carry out such work as importing, digesting, and absorbing technology and conducting intermediate experiments. Our country's large- and medium-sized industrial enterprises and enterprise groups should play a key role in making technological progress.

--The original treatment and status accorded to the research institutes entering large- and medium-sized enterprises or enterprise groups should be preserved for some time. In so doing, we can relieve the institutes of their worries for the immediate future and allay the worries of the enterprises which want the institutes but are afraid that they cannot afford them. In a few years time, after the enterprises have enormously strengthened themselves, increased their vitality, and attained the capability to more satisfactorily support the technological development institutions to play their role, the research institutes will be able to dispel their misgivings. At that time, we shall be able to give them a free hand.

--Efforts should be made to enable both the enterprises and research institutes to derive greater material benefit after the association than before. This chiefly means that, on the one hand, the research institutes entering enterprises or enterprise groups may obtain funds from the enterprises; on the other hand, the state will, for a long time and through the original channel, allocate the same amount of money to the institutes as the operation funds allocated the year before they enter the enterprises. The enterprises absorbing the research institutes will also be allowed to enjoy the tax-free preferential treatment for income which the original research institutes received from the transfer of technological inventions to other units.

Nationally, there are so many types of research institutes that we should deal with different things in different ways. There should not be only one pattern. In our view, however, it is necessary to explicitly guide most institutes for technological development, and especially the product research institutes, to form close associations with enterprises, preferably to gradually enter enterprises or enterprise groups.

Question: Please explain the reasons for implementing, and the principal content of, the policy for relaxing controls on scientists and technicians.

Answer: It is pointed out in the "State Council Decisions on Further Carrying Forward the Reform of the Science and Technology Management System" that it is necessary to further reform the scientists and technicians' management system and to relax the policies on scientists and technicians so as to create a favorable social environment for them to give full play to their role. This is a very important decision.

Reforming the scientists and technicians' management system is a task explicitly laid down in the central decision on reforming the science and technology management system. In the past year and more, all localities have done a lot of work and have made initial progress in various aspects, such as gradually instituting the system of employing people for special technical posts, promoting the rational flow of scientists and technicians, and implementing the policy of rewarding scientists and technicians who have made contributions.

However, the systems developed over the years set excessive restrictions on scientists and technicians. Insufficient tasks make it impossible for the scientific research institutions, institutes of higher learning, and other units, where scientists and technicians are concentrated in large numbers, to give play to the role of their personnel. Meanwhile, the textile and other light industries, commerce, the vast rural areas, and towns are very short of highly-trained people and technology. The large numbers of small- and medium-sized enterprises and town and township enterprises occupy an important position in the national economy. The enormous natural resources and manpower there can genuinely turn into actual wealth only when there are highly trained people and technology. For this reason, it is necessary to relax the policies still further in order to encourage and attract scientists and technicians to work there.

The "decisions" support and encourage scientists and technicians in scientific research institutions, institutes of higher learning, and government offices to go to the rural areas, small- and medium-sized cities and towns to spread science and technology, to run enterprises under a contract or leasing scheme, to set up various institutions for technological development and services, as well as small- and medium-sized joint ventures of various types, and so on. In short, we should let them give full scope to their talents in revitalizing the economy and developing science and technology, do pioneering work, and perform meritorious deeds for the people. The vast rural areas and the cities and towns urgently need a large number of people who can lead the people to prosperity. They also need to train through practice a contingent of new entrepreneurs and businessmen who understand technology.

Scientists and technicians can go to enterprises by various means. They can be selected by the relevant departments, regions, or units and sent to enterprises to take up various posts. They can also run small- and medium-sized enterprises under a contract or leasing scheme. The relevant units become shareholders, draw dividends, and share risks by supplying technology, funds, and equipment. They can also contract for enterprises individually or in groups. Those applying for leave without pay should have the approval of the respective units, which should set a time limit for their leave to enable the scientists and technicians to adapt themselves to work in the rural areas or in towns. During their leave, they should turn over some money to the units and, at the expiration of their leave, should either return to the original units to work or hand in their resignation. The relevant units should support those who

voluntarily resign to run enterprises in town and rural areas. Those who resign should hand in their applications 3 months in advance and should go through handing-over and resignation procedure before they leave. However, they should not leave without authorization on the pretext of "relaxing policies on scientists and technicians." Still less should they infringe upon the rights and interests of the original units by privately taking away the technological inventions or restricted data of the units.

The "decisions" explicitly point out that it is necessary to let those who lead the people to prosperity become prosperous themselves. Their income from operating enterprises under a contract or leasing scheme and setting up institutions for technological development and services, as well as joint ventures, is not restricted by their original pay. Scientists and technicians should be allowed to become shareholders by providing technology and to draw dividends according to the number of their shares. If their income reaches the level of regulatory tax, they should pay taxes according to the stipulations. The relevant departments should properly help scientists and technicians remove their worries for the future and protect their legitimate income.

In relaxing policies on scientists and technicians, it is also necessary to vigorously unclog the channels for the flow of highly trained people, to strengthen the lateral links between the various departments of the State Council and the various provinces, municipalities, autonomous regions, and key cities, and to forge closer contact between the unit employing such personnel and the unit they formerly belonged to.

Relaxing policies on scientists and technicians will speed up the commercialization of technological inventions, promote the development of new technologies and industries, and bring up a large number of new entrepreneurs and businessmen for the development of the socialist commodity economy and scientific and technological undertakings.

In fact, the nongovernmental scientific and technological institutions that have emerged in the course of reform practice have become a force to be reckoned with in China's scientific and technological ranks. They also serve as an important supplement to the exisiting scientific and technological institutions. For example, without any investments from the state, the Beijing Stone Group Corporation, which was set up by a dozen or so young and middle-aged scientists and technicians in May 1984, has built itself up by relying on some 20,000 yuan borrowed from other people. In 2 and 1/2 years, it has developed into a nongovernmental scientific and technological development institution with 270 employees, 1 million yuan worth of fixed assets, more than 100 million yuan in output value, and more than 10 million yuan in profit. With an annual per capita output value of over 300,000 yuan and an annual output growth rate of 300 percent, Stone is incomparably superior to the ordinary state-owned scientific and technological institutions. The strong points and vitality demonstrated by nongovernmental scientific and technological institutions have provided important inspiration to the advance of reform.

To encourage scientists and technicians to become entrepreneurs and businessmen of a new type, it is necessary to advocate in the whole society a conscientious change in concepts. It is particularly necessary to overcome the ideas or concepts of hierarchy of feudal literati and officialdom and foster the idea that it is a glorious thing to be oriented toward the economy and to yield better results.

/12858
CSO: 4008/2104

ZHOU GUANGZHAO ON S&T ACHIEVEMENTS

OW101254 Beijing XINHUA in English 1236 GMT 10 Mar 87

[Text] Beijing, 10 March (XINHUA)--The Chinese Academy of Sciences completed 1,411 research projects in both applied and pure science last year, according to Zhou Guangzhao, president of the academy.

"Two-thirds of these achievements were related to key projects in the national economy," Zhou said at a meeting here today.

"Most of these achievements are up to international standards and some are even leading the world," he noted. For example, the technology and material used to build an undersea robot has withstood pressure 199 meters beneath the sea.

The academy is also entering the international market with its new technology and products. High-quality artificial crystals developed by Fujian Institute of the Structure of Matter and Shanghai Silicate Institute are highly valued by the international scientific community and are selling on American, Japanese, and European markets.

A breakthrough in superconductors by the Physics Institute has had a strong impact abroad. Also, "The Geochemistry of Strata-Bound Ore Deposits of China" compiled by the Guiyang Institute of Geochemistry has considerable theoretical and application value.

Last year the academy also won five gold medals at international invention and new technology fairs held in Geneva and Brussels.

/8309
CSO: 4010/2018

ZHOU GUANGZHAO ON BASIC RESEARCH BODIES

OW101814 Beijing XINHUA in English 1540 GMT 10 Mar 87

[Text] Beijing, 10 Mar (XINHUA)--In a bid to change its fundamental structure, the Chinese Academy of Sciences [CAS] has proposed setting up new bodies for basic research.

According to President Zhou Guangzhao, some of the CAS's existing institutes and major scientific projects will become national laboratories or scientific centers administered by scientists themselves and open to foreign scientists.

President Zhou urged institutes to set up joint laboratories and other cooperative projects with colleges and universities, and also encouraged institutes to seek overseas support and cooperation to become international research centers.

The new research bodies will be competitive internationally in major and long-term research in the basic sciences, Zhou said. "We will follow an open policy, by making efficient use of scientific resources and promoting academic exchange."

The CAS was founded in 1949 and currently has 122 research institutes employing 80,000.

/8309
CSO: 4010/2018

MACHINE BUILDING INDUSTRIES IN FOUR AREAS DESCRIBED

Guizhou Industry

Beijing ZHONGGUO JIXIE in Chinese No 1, 25 Jan 87 pp 25, 27

[Article by Shen Liansheng, Director of Guizhou Machine Building Industry Bureau]

[Text]

Guizhou Province, located at the east of Yunnan and Guizhou Plateau, with total area of 170,000 square kilometers and a population of over 28 million, is a place where multiple nationalities are living together. Our province is situated in subtropical zone with abundant rainfall and rich energy and mineral resources but without the intense heat of summer and severe cold of winter. Ther reserves of mercury, optical crystal and high quality phosphorous rank first in the whole country and bauxite the second, all of which provide very favourable conditions for the development of machine building industry and foreign trade of our province.

At present, our province has built a complete machine building industry consisting of agricultural machinery, construction engineering machinery, machine-tools and toolings, optical instruments, electric meters and instruments, electronic and electric apparatus, general machinery, automobiles, bearings, grinding materials and abrasives as well as civil building machinery, cereal, oil and food processing machinery, food packaging machinery and medical and hygeian apparatus, etc. with a great number of key enterprises of good quality and high production level, producing more than 2,000 varieties of machinery products. For example, the hydraulic excavator manufactured by Guiyang Mining Machinery Plant was awarded State Silver Medal; and the "Tianli" Brand diesel engines, Model 6135-AD, AG produced by Guizhou Diesel Engine Plant are the products honoured with the State Silver Medal and have been exported far away to more than 40 countries and regions. Xintian Fine Optical Instrument Corporation is one of the five production basis of precision measuring and testing instruments in China. The precision optical metrology and physical instruments such as 3-dimensional measuring instrument, polarizing microscope, universal toolroom microscope manufactured by this Corporation enjoyed a certain reputation both at home and abroad. Changzheng Electric Apparatus Corporation is one of the five low-voltage electric apparatus production basis in China producing various low voltage electric apparatus, high voltage load voltage-regulating switches and electric control complete plant installations provided for the large and medium sized electric transmission and substation projects, high rank hotels,

machine tools and diesel generating sets. The centerless grinding machine, roller grinding machine, rolling and pressing machine and large precision gudie-way grinding machine made by Xianfeng Machine Tool Works enjoyed a good reputation both at home and abroad. Micro-bearings and aircraft engine bearings produced by Hongshan Bearing Factory occupied a certain position in domestic and foreign markets. The rich bauxite, coal and electricity resources in our province created very favourable conditions for the development of grinding materials and abrasives industry. Three of the six largest grinding wheel factories in China are in our province. Their main products include brown and white corundum, green and black silicon carbide, artificial diamond, cubic boron nitride and their finished products. The abrasives "Mountain" Brand brown corundum made by The Seventh Grinding Wheel Factory were awarded State Silver Medal for its quality and enjoyed higher reputation both at home and abroad and have been exported to Japan and U.S.A.

Under the guidance of the policy of "opening door to foreign countries and taking flexible way in China" by our government, our province is working hard at the development of economic and technical cooperation with foreign countries, planning to import 50 items of advanced technologies from foreign countries for remoulding over 100 main manufacturers, developing mainly 200 varieties of products such as electric melting aluminium oxide, aluminium corundum, high quality super-hard materials and its finished products, large NC cutting machines, CNC machining centers, precision guide-way grinding machine, load separately-connecting switches, leakage protection switches, 3-dimensional measuring instrument, high rank polarizing microscope, hydraulic excavators and various modified automobiles etc., all of which will make Guizhou machine building industry develop into a new stage.

With beautiful mountains and rivers, mild weather and rich resources, Guizhou has always enjoyed a reputation of being a "famous scenic spot in Qianzhou". It is also famous both at home and abroad for its marvelous and grand Huangguoshu Waterfall and under ground dragon cave.

Quizhou is one of the developing economic zones in China and also an ideal place for the realization of great plan for the wide cooperation with foreign friends. In the ever prosperous economic activities, we warmly welcome economic and trade businessmen from different countries in the world, our compatriots in Hong Kong and Macao and overseas Chinese to continuously expand mutual business intercourse and economic & technical cooperation, establish long term coexisting and stable developing trade relationship, increase mutual friendship and strengthen sincere cooperation on the base of mutual benefit and exchange of mutual needs and make helpful contributions for the great development of economy and trade and for the promotion of friendly contact among different countries.

Welcome to our province for visiting and sight-seeing.

Welcome to our province for business negotiation.

Shaanxi Industry

Beijing ZHONGGUO JIXIE in Chinese No 1, 25 Jan 87 p 27

[Article by Liu Jiantang, Director of Shaanxi Machine Building Bureau and Du Kejun, Chief of the Research Office, Shaanxi Machine Building Bureau]

[Text]

After more than 30 years' construction, the machine building industry in Shaanxi Province has built up its manufacturing system with certain scale and technical level and has become one of the main industrial sectors in the province. In order to suite the needs of economic development, build up machinery industry with our own characteristics, speed up technical reform, strengthen its competitive capability at home and abroad, increase economic benefit and foreign currency income, we are willing to have economic and technical cooperation in various fields with foreign firms on the basis of mutual benefit and development.

We have favourable situation for investment in machinery industry.

1. Fairly production basis. There are dozens of big, medium-sized enterprises with sufficient equipment.

2. Rational distribution of industry. Most of the enterprises are scattered in the Qinchuan plain and along the railway of Longhai, centered in Xi'an, Baoji, Xianyang, Tongchuan and Hanzhong Basin. It is convenient for ally and coordinate each other.

3. Good industrial structure. It mainly serves energy resources, communication, raw materials and agriculture.

4. Rich in energy. Shaanxi Province has enough coal and power resources which are very important for the development of machinery industry.

Compare with sea coast areas, the province enjoys exceptional advantages.

5. Powerful forces in scientific research. The province has two advantages which are big military service factories with high level technique and advanced test method as well as quite a number of specialists and professors in the engineering collages and institutes.

6. Convenient communication. Highway, railroad and air routes link up all parts of the country.

7. Eager desire of incorporation of advanced technology and experience.

8. Benefitial in policy.

In the coming period, the province will put stress of investment for machinery industry on:

A. Development of power industry. Extra high voltage A.C. and D.C. power transmission equipment will be main items for reform.

B. Improvement of conveyance level of automation meters.

C. Improvement of the accuracy of precision machine tools and automation. Pick up speed of organic whole of mechanical and electrical products.

D. According to the development of agriculture, produce and supply the market with suitable agricultural machinery.

E. Develop products which have reputation inside China with characteristics of Shaanxi Province, such as bulldozers, forklifts, precision machine tools, heavy duty automobiles, heavy duty forging equipment, precision bearings, printing machinery and turbine devices for generating by surplus heat from furnaces.

In order to bring the existing and potential superiority of the provincial machinery industry into full play by importing high level technology and equipment as well as ally with the same trade or occupations at home, we will build up a manufacturing system in the machine building industry with electrical equipment, instruments and meters, precision machine tools, heavy duty automobiles heavy duty mining machinery as its 5 leading basis in Shaanxi Province.

Shandong Industry

Beijing ZHONGGUO JIXIE in Chinese No 1, 25 Jan 87 pp 28-29

[Text]

Shangdong, one of the ten coastal provinces and municipalities in China with two open cities and five open ports to foreign countries, has established economic and trade relations with more than 140 countries and regions in the world. The two cities, Qingdao and Yantai are the economic developing zones which have preliminaryly provided with the environment for absorbing foreign investment and created excellent conditions for the machine building industry to import technology, utilize foreign capital and expand export.

Since the founding of the People's Republic of China, Shangdong Machine Building Industry has developed very quickly and it has already set up a dozen industries such as automobile, machine tool, farm machinery, electric apparatus, ship, heavy mining, construction engineering machinery, packaging and food processing industries etc. Its industrial production value in 1985 ranked the fourth in the whole country. With solid foundation, it has set up a great number of key enterprises and developed many important products such as heavy trucks, machine tools, forging presses, foundry machineries, small generating sets etc. which occupied leading position in domestic market. Its actual strength is much stronger in production of farm machinery with more serialized products. The products of this province has occupied a certain position and enjoyed higher reputation in the international market. Shaping machines, Mazak lathes, abrasives and grinding tools, "Mount Taishan" Brand tractors of 25 HP and crushers have been well received by our foreign customers.

Shangdong possesses rich natural resources and a larger reserve of different ores. The development and utilization of the raw materials such as iron, coal and oil etc. as well as energy have not only set a new demand on engineering products, but also provided with reliable material guarantee for further development of machine building industry.

The main development target during the period of the "Seventh Five-Year Plan" is to make the main and export products come up to international standards, develop new products and renew 1,500 varieties of old products, and up to 1,990, making 50 percent of the main products reach the international advanced level of the end of 1970s and the beginning of 1980s. The production of important products and engineering products urgently needed by the state will be expanded through technical remoulding of the original enterprises and importation of advanced technology from foreign countries. A greater development in production of heavy, medium and high load trucks will appear. The important point in electric apparatus industry is to organize the production and supply of the complete plant of small generating sets and develop large transformers and second generations small and medium-sized energy-saving transformers. The main task in machine tool industry is to develop precision, high efficiency and mechanically and electrically integrated products, greatly raise accuracy and repeatability of products. In farm machinery, the most important thing is to change structure of products and increase new varieties and various modified models. Packaging and food processing machine building industry is a rising industry which is planned to set up gradually a large number of specialized manufacturers.

Shangdong Machine Building Industry will have a greater development. We would like to support each other with our brother provinces, go forward hand in hand and strengthen relations and wide cooperation with our customers both at home and abroad.

Inner Mongolia Industry

Beijing ZHONGGUO JIXIE in Chinese No 1, 25 Jan 87 p 30

[Article by Lu Huannan]

[Text] The Inner Mongolia Autonomous Region is the earliest one being founded as a national minority autonomous region in the People's Republic of China. After the liberation of the country, the industry in this region grew out of nothing and developed rapidly. At present, an essential scale of industry is established. By the end of 1985, there were 1767 machinery manufacturers and the value of output is 1.75 billions RMB, about one fifth of the total industrial value in this region. The value of fixed assets is more than 2 billions RMB. There are more than 40 thousands of machine tools for metals and more than 8 thousands of forging and pressing equipments. The value of profits and duties together is 230 millions RMB. The machinery industry is one of the major industrial mainstay in this autonomous region.

Since the Thrid Plenary Session of the Eleventh Central Committee of the Party, the principle of "three uprises and one increase" (i.e. uprises of quality, variety and level and increase of economical benefit) issued by the Ministry of Machine Building Industry has been implemented attentively. To conduct the modernization and to promote the combination of enterprises causes the industry full of vitality. The animal husbandry machinery in this region possesses distinctively local features, and occupies a dominant position in the country. Machineries, such as trailed mower, trailed rake, animal drawn mower, straw chopper, hey tedder, milker, forage harvester, small wind energy system, electric fence, win praises by the people broadly inside and outside of this region. Especially, the manufacture of small size wind energy system is in the leading position in the nation. In 1985, there were 15 thousand units been produced and a new industrial line of wind energy is formed. By implementing the policy in economy of "forestry and animal husbandry to be developed", the Inner Mongolia Autonomous Region decides to develop animal husbandry machinery industry with effort, to give major support in production planning and with raw materials, to develop famous products with good quality, to serve the region and the country, and to strive to market abroad.

The manufacture of general machinery in this region has also reached a definite level. Manufacturing hundreds kinds of machineries such as machine tools, tractors, refitting automobiles, bearings and various kinds of parts and components can be done. Certain products have been approved as "Superior quality Product" either by the State, the Ministry of Machine Building Industry, or by the Autonomous Region. For example, the Universal triple clamp, produced by Huhehot Machine Tool Accessory Works has been named "Trustworthy Product" by foreign trade departments, being exported to more than 40 countries and areas. The export quantity of this product is about 80% of the total export quantity of clamp in our country. The electrode produced by Jining Electrode Works is famed abroad, and has concluded a foreign trading of nearly two thousands tons of products during the Spring Guangzhou Commodity Fair in 1986. The insulating materials produced by Baotou Insulating Material Factory are also popular in international market. The products such as motors, transformers, wire and cable have changed to there new generations and the supplying talls short of demands in market. The big truck for mines, railway rolling stocks hydraulic frame, beam pumping unit, electric meters and so forth are also popular to the end-users. Along with the horizontal economical combination among enterprises, there must be more and more new machinery products. The machinery industry in Inner Mongolia is developing day by day, with a very bright future.

/9317
CSO: 4010/2014

ADVANCES IN CASTING TECHNOLOGY REPORTED

OW071242 Beijing XINHUA in English 1041 GMT 7 Mar 87

[Text] Beijing, 7 Mar (XINHUA)--A female professor's research has improved China's casting technique and helped put China's technology of casting key parts for plane and ship engines into the forefront worldwide.

Zhang Litong, of the Xi'an-based Northwest Engineering University, has devoted a decade to the research to develop China's ancient "lost-wax" casting technique and successfully worked out her own system of investment casting.

The system is a great contribution to China's precision casting industry by greatly improving China's technique for making vanes, the key parts of jet engines for aircraft and ships.

China was one of the earliest inventors and users of lost-wax casting for forming metal shapes 2,400 years ago. Its main processes are like this: a die-mold is formed with wax; the waxed mold is used to form the shell of the metal shape; the wax is melted to leave a finally-processed mold; and the molten metal is poured into the mold for the final product.

However, the technique was developed further in foreign countries in the ensuing centuries and the most advanced method was used to make the engine vanes for the Boeing-767 aircraft.

In 1976 when it tried to import an assembly line to produce airplane engines, China had to spend millions in foreign exchange to buy the patent. This made Zhang think a lot and put her heart into the research.

Zhang has been honored as "a state-class scientist who has made an outstanding contribution to the country" by the State Science and Technology Commission.

/8309
CSO: 4010/2018

HIGH-PRESSURE CONTAINERS FOR CHEMICAL INDUSTRY

OW261946 Beijing XINHUA in English 1602 GMT 26 Feb 87

[Text] Beijing, 25 February (XINHUA)--Two Chinese factories have been granted certificates of quality by the American Society of Mechanical Engineers for specialized containers, according to an official from the Ministry of Chemical Industry.

The Nanjing Factory of Chemical Machinery and the Dalian Jinzhou Factory of Heavy Machinery received the certificates for their high-pressure containers, an important piece of equipment used in the chemistry and chemical petroleum industries, the official said.

The containers are made to resist high or low temperature, pressure and corrosion, the official said.

"The certificates prove that China's technology for making such containers has entered a higher level," the official said.

Unable to produce large high-pressure containers for the chemical industry before the 1970s, China had to import them from abroad, the official noted.

Since 1981, China has gradually been able to produce large containers and put an end to imports from foreign countries. In 1983, China produced a 320-ton, 2.8-meter in diameter container, he said.

The American Society of Mechanical Engineers has offered certificates to qualified producers of pressure containers in all countries since 1972. Container makers around the world consider the certificate as a mark of quality, the official said.

/8309
CSO: 4010/2018

DETAILS OF BIG SH5 FLYING BOAT LISTED

Beijing GUOJI HANGKONG [INTERNATIONAL AVIATION] in Chinese No 3, Mar 87 pp 31, 24

[Excerpts] The "SH5" is a first-generation flying boat developed by China in the 1970's. It was developed in response to a Navy request to beef up sea defenses. The aircraft is a four-engine maritime bomber that can perform both military and civilian missions. From the raw materials to the assembly of the final product, this was a purely Chinese research and development effort involving some 100 factories, institutes, and schools. Its successful development proves that China is not only capable of producing all types of fighter aircraft, bombers, helicopters, military and civilian transports and passenger aircraft, but also large, technically complex flying boats.

Work on the design of the SH5 was begun by the Ministry of Aviation Industry's Flying Boat Research and Design Institute in April 1969. The first experimental aircraft was produced at the Harbin Aircraft Manufacturing Corporation and rolled out of their plant in 1971. In 1971, the aircraft was subjected to 110-percent design load tests and destructive tests. Also, many wind tunnel tests were conducted as well as high-speed towing experiments in a water basin. Data from the verification of the test flights demonstrated that the results of the wind tunnel and basin tests were correct and reliable. The aircraft's four powerplants were all-new turboprop engines developed by the Harbin Aero-engine Company. Each engine is a 3,150-horsepower WJ-5A. The airscrew is designated "J19-G10" and has four blades. The research and development of the aircraft's radar, various electronic equipment and systems as well as the associated machinery was a coordinated effort by domestic research institutes and plants that enabled the aircraft to make its initial flight on 3 April 1976.

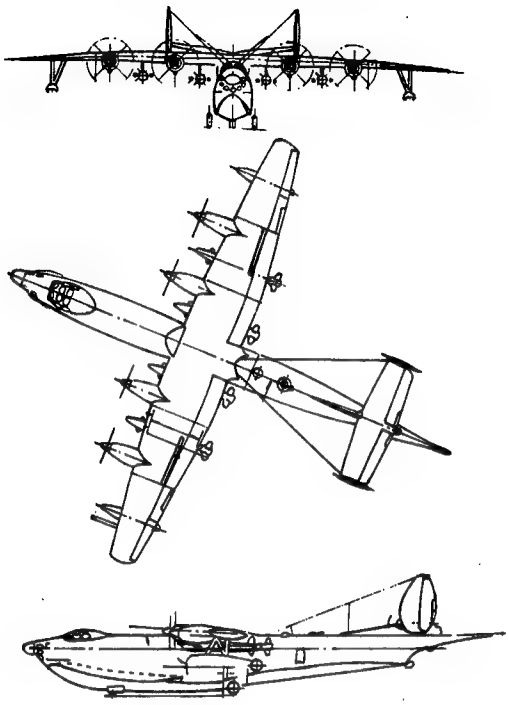
The various flight tests and trials have proven that the SH5 is manoeverable and safe at low altitudes. Water-tight riveting is employed on the lower hull and heat- and corrosion-resistant materials and coatings have been used throughout the aircraft, which reflects extensive use of new techniques and technologies. The WJ-5A power plant can still produce 3,150 hp at tempetarures of 30°C. The four-bladed J19-G10 prop is fitted with de-icing equipment and has feathering and reverse-pitch capability. The control systems employ an autopilot and three-axis compound hydraulic movable surface power units as well as pneumatic and manual systems. In order to assure that the aircraft can start its engines by itself at any time while on the water, it is equipped with a

motive power system. In addition, the aircraft is equipped with an inertial guidance system, attitude [control] systems, a meteorological data center, ultrashort wave radios, single sideband radios, radio altimeters, radio compasses, ultrahigh frequency directional antennas, and Doppler radar. The military version can also carry a variety of weapons systems such as antishipping missiles, antisubmarine guided torpedos, conventional bombs, depth charges, aerial mines, as well as sonars, magnetic detectors, etc.

The principal roles of the SH5 are passenger service, cargo transport, rescue, exploration, survey, and development of ocean resources, weather reconnaissance, maritime patrol, environmental protection, fighting forest fires, and coast defense.

Technical data:

Wing span	36 meters
Length	38.9 meters
Height	9.79 meters
Weight empty (anti-submarine role)	26.5 tons
(transport and rescue role)	less than 25 tons
Normal take-off weight	36 tons
Maximum flying weight	45 tons
Fuel capacity	16.5 tons
Maximum speed	555 kilometers/hour
"Minimum" speed	230 kilometers/hour
Cruising speed	450 kilometers/hour
Take-off speed	160 kilometers/hour
Landing speed	170 kilometers/hour
Service ceiling	7,000 meters
Maximum range	4,750 kilometers
Maximum sustained flight time	12-15 hours
Wave resistance	2.1 meters
Take-off distance on water	548 meters
Landing distance on water	240 meters



/8309
CSO: 4008/44

REVISING PROPOSAL TO THE RECOMMENDED MEASUREMENT METHOD OF ERRORS OF PAL CHROMINANCE SIGNAL DEMODULATION ANGLE

Tianjin TIANJIN DAXUE XUEBAO [JOURNAL OF TIANJIN UNIVERSITY] in English, No 1, Jan 87 pp 115-126

[English abstract of article by Yu Sile [0205 2448 2867] and Li Guiliug of the department of Electronic Engineering]

[Text] This paper analyses the principles of the recommended method for measuring errors of PAL chrominance signal demodulation angle, which is constituted in IEC [Production and Application of Light] Publication 107-1[1]. Analysis shows that by means of above method, the measured phase matching error and amplitude matching error of delayed and undelayed signals are mixed with other errors. Thus the measurement results, especially for phase matching error, cannot correctly reflect the errors in associated circuits and cannot be used for assessing the properties of the comb filter or the receiver. In this paper, authors propose that the measuring signal and the measured point in the receiver should be changed. Practical measurement and observation of the line crawl on screen show that the modified method is more effective and more reasonable. (Paper received 2 September 1985.)

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/6091
CSO: 5500/4153

BRIEFS

CAS EARNS FEX--Beijing, 10 March (XINHUA)--The Dongfang Company, affiliated with China's Academy of Sciences, earned US$15.6 million last year. The CAS's foreign currency earnings increased 160 percent over 1985, and export-oriented enterprises and departments increased from 35 to 47, with seven pulling in more than US$100,000 last year. Products like electrical equipment, components and parts earned only US$74,000 in 1980, but last year these same products tallied US$5.09 million. A number of China's hi-tech and name brand products have made their way to international markets and are being well-received by customers. [Text] [Beijing XINHUA in English 1303 GMT 10 Mar 87] /8309

TECHNOLOGY IMPROVES EFFICIENCY--Beijing, February [no day] (XINHUA)--By 1990, China will have introduced another 70 new technological procedures to improve its industrial efficiency, today's ECONOMIC DAILY reported. These include a rust removal method expected to save 500 million yuan (135 million U.S. dollars) by 1990, as well as methods to brew beer and wine from corn and use water to replace oil as a lubricant. During the Sixth Five-Year Plan (1981-1985), 40 new technological procedures were implemented in China. Thanks to this, China's nonferrous metallurgical industry produced an additional 76,000 tons. [Text] [Beijing XINHUA in English 0712 GMT 27 Feb 87] /8309

MNI ADVISORY CENTER IN JINAN--The Ministry of Nuclear Industry formally established an advisory center of scientific and technological services in Jinan City, Shandong Province, on 4 March. Ma Shizhong, vice governor of the province, and responsible comrades concerned from the Ministry of Nuclear Industry attended the inaugural meeting. The service center will be regarded as a window of scientific research and production combination between the province and the ministry, carry out various business activities, popularize or transfer the new civilian-oriented technology and products offered by the ministry, exchange information, conduct technical consultations, train talented personnel, organize the technology of jointly increasing the variety of new products and the funds of jointly opening plants, and will take up the exhibition sales of new products offered by the ministry. During the inaugural meeting of the service center, the ministry also held trade talks on scientific and technological results, at which the departments concerned under the ministry signed the contracts on new technological transfer with the Linyi Prefecture of Shandong Province and the (Luxiang) District of Jinan City. [Text] [Jinan Shandong Provincial Service in Mandarin 2300 GMT 4 Mar 87 SK] /12858

CSO: 4008/2104

GENERAL EQUATIONS FOR 3-DIMENSIONAL COMPUTER SIMULATION OF LOW-PRESSURE CHEMICAL VAPOR DEPOSITION

Beijing ZHONGGUO KEXUE (A JI) [SCIENTIA SINICA: SERIES A (MATHEMATICAL, PHYSICAL, ASTRONOMICAL & TECHNICAL SCIENCES)] in Chinese No 11, 1986 pp 1213-1222

[Article by Wang Jitao [3769 1323 7118] and Zhang Shili [1728 0013 3810] of Department of Material Science, Fudan University, Shanghai]

[Abstract] In the axial and diametral directions of the reaction tube using the inversion rates η and $\eta(\rho, \pm\ell)$ for the molecules of an introduced reaction gas, respectively, into sheets and between the sheets, and also using the transport shunting factor ξ of the reaction gas, general equations for the three-dimensional computer simulation of low-pressure chemical vapor deposition (LPCVD) were proposed. These equations can simultaneously simulate computing of various homogeneities between and in the sheets of LPCVD. The computation results match quite closely with the experimental results. The effect of gas diffraction flow, Bernoulli effect, adsorption of reactant molecules, and desorption of reaction by-product molecules on the equations were discussed; in addition, appropriate revisions were made. These are the major advantages for the computer simulation equations for three-dimensional LPCVD: simple expressions, universality not restricted by the number of chemical reaction levels, rapid computation rate, and small internal memory needed in the computer, among others. Hence, the derivation of the three-dimensional LPCVD computer simulation equations facilitates the execution of microcomputer control of LPCVD thin film technique.

One table lists the parameters of commonly used reaction systems. Eleven diagrams show the theoretical model and reaction cavity for simulation computation; comparison with Rosler's experimental curve; effects of total gas flow, reaction gas concentrations, deposition temperature, total system pressure, inter-sheet gas, and clearance from the inner wall of reaction tube to the edge of silicon lining base for the in-sheet homogeneity, and their experimental results.

The first draft of the paper was received on 10 January 1984; the final, revised copy was received for publication on 18 December 1985.

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10424/6091
CSO: 4009/1052

PREDECESSOR k-NEAR NEIGHBOR COMPILING DESIGN METHOD OF TREE CLASSIFIER

Beijing ZHONGGUO KEXUE (A JI) [SCIENTIA SINICA: SERIES A (MATHEMATICAL, PHYSICAL, ASTRONOMICAL & TECHNICAL SCIENCES]) in Chinese No 11, 1986 pp 1223-1232

[Article by Wang Qingren [3769 1987 0086], Department of Computer and System Science, Nankai University, Tianjin]

[Abstract] The compilation near-neighbor (NN) method in non-parameter pattern classification leads to a near-optimal recognition rate that depends on probability; however, the machine time and internal memory used are O(n), and n is the volume of training samples. The tree classifier can reduce the machine time used to O(log n). However, all designs of the present tree classifier first classify the space before compiling the training samples, thus hampering a gain in the recognition rate. Generally, these studies do not estimate the corresponding near-optimal recognition rate. The paper presents a design scheme for a tree classifier where first the samples are compiled and then the space is classified in describing the probability of a near-optimal recognition rate, and in presenting a larger scale computer simulation test. As indicated by the test results, this tree classifier has a high recognition rate, as well as high time and space efficiencies. In the contrast test with n = 3200, this method is faster by 1,000 times than the k-NN method and the compilation NN method, and faster by 156 times than the concentrated NN method.

Four diagrams show the post-compilation Voronoi graph and the compilation-NN classification boundary plane, tree classifier, decisionmaking boundary plane of the predecessor compilation tree classifier, and the probability distribution of simulation data. Six tables list mean error rates and the volume of examined samples of the k-NN classification, the CPU time used in k-NN classification, compilation and concentrated NN classifiers, and the predecessor compilation tree classifier.

The first draft of the paper was received on 24 September 1985; the final, revised draft was received for publication on 11 May 1986.

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CSO: 4009/1052

STUDY OF MAGNETIC STABILITY OF METALLIC GLASS $(Fe_{0.1}Ni_{0.33}Co_{0.55}Cr_{0.02})_{78}Si_8B_{14}$-- II. IRREVERSIBLE ATTENUATION OF MAGNETOCONDUCTIVITY OF DEMAGNETIZATION

Beijing ZHONGGUO KEXUE (A JI) [SCIENTIA SINICA: SERIES A (MATHEMATICAL, PHYSICAL, ASTRONOMICAL & TECHNICAL SCIENCES)] in Chinese No 11, 1986 pp 1182-1188

[Article by Zhang Yanzhong [1728 1693 1813], Shanghai Institute of Iron and Steel]

[Abstract] In the 100 to 300°C range, the equal-time magnetoconductivity attenuation was measured. The dynamic behavior of attenuation was examined, and the distribution of excitation energy in the attenuation process was estimated. Thus, the author recorded the attenuation spectrum, coercive force and core wearing of single peaks since the peak positions are closely spaced between the Curie temperature relaxation spectrum and the magneto-conductivity attenuation. The author examined the relationship between magnetoconductivity attenuation and induced magnetic anisotropy of the annealing procedure, and the observed reversibility of magnetoconductivity attenuation and the Cross-Over effect.

Nine diagrams show the relationship among magnetoconductivity attenuation, induced anisotropy and annealing temperature, magnetoconductivity attenuation dynamic curves, activation energy spectrum, equal-time relaxation spectrum, variation of magnetoconductivity during cyclic annealing, and the Cross-Over effect. The first draft of the paper was received on 10 August 1985; the final, revised draft was received for publication on 18 May 1986.

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10424/6091
CSO: 4009/1052

COUPLING OF BOUND EXCITONS AND PHONONS IN GaP:N

Beijing ZHONGGUO KEXUE (A JI) [SCIENTIA SINICA: SERIES A (MATHEMATICAL, PHYSICAL, ASTRONOMICAL & TECHNICAL SCIENCES)] in Chinese No 11, 1986 pp 1175-1181

[Article by Zheng Jiansheng [6774 0256 3932] and Zhang Yong [1728 0516] of Department of Physics, Xiamen University]

[Abstract] Using the photoluminescence approach, the paper studies the coupling of bound excitons and phonons in GaP:N with different blended N concentrations in the 15-80°K range. As revealed by the experimental results, the same temperature dependence exists for the zero phonon line and the corresponding phonon accompanied lines; under the Huang-Rhys multi-phonon light skip theory, the various S factors (Huang-Rhys factors) do not vary with temperature. Two tables list energy data of various phonons and S factors. Seven diagrams depict a low temperature photoluminescence experimental system, the photoluminescence spectrum of bound excitons, and the relationship between temperature on the one hand, and S and K, on the other. The authors are grateful to Professors Wu Boxi [0702 0130 0296] and Liu Shiyi [0491 1102 3015] for their support of the research. The first draft of the paper was received on 17 April 1985; the final, revised draft was received for publication on 9 December 1985.

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CSO: 4009/1052

STEPWISE EXCHANGE ISOTHERM OF COPPER, ZINC, CADMIUM LIQUID-SOLID PARTITIONING ON γ-Al_2O_3 in SEAWATER

Beijing HAIYANG YU HUZAO [OCEANOLOGIA ET LIMNOLOGIA SINICA] in Chinese Vol 18 No 1, Jan 87 pp 76-85

[English abstract of article by Zhang Zhengbin [1728 2973 2430], et al., of Shandong College of Oceanology, Qingdao]

[Text] 1. This paper deals with a new type of isotherm in seawater systems. It has a characteristic of the isotherm being made up of two "S shaped" curves, which intersect each other near the middle with one "knee" and two "plateaus" at the intersection. The order of the first saturated quantity of Cu, Zn and Cd on γ-Al_2O_3 is:

$$Cu > Zn > Cd$$

2. In order to explain theoretically the new isotherms, this article suggests the application of interfacial stepwise ion exchange for liquid-solid distribution of minor elements on suspended particulate matter to derive a corresponding isotherm equation:

$$\theta = \frac{\sum_{i=1}^{N} i\mathscr{K}_i a_M^i}{1 + \sum_{i=1}^{N} \mathscr{K}_i a_M^i}$$

let i - 1, 2, then we have:

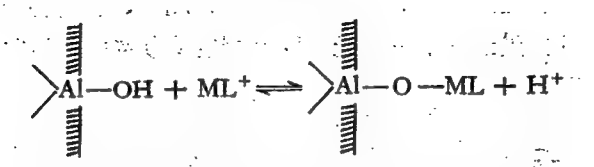
$$\frac{\theta}{(1-\theta)a_M} = \mathscr{K}_1 + \left(\frac{2-\theta}{1-\theta}\right) a_M \mathscr{K}_2$$

The authors suggest using the extrapolation method and plot "$\frac{\theta}{(1-\theta)a_M} - \frac{(2-\theta)a_M}{(1-\theta)}$ graph." $\mathscr{K}_1$ and $\mathscr{K}_2$ can be obtained from the intercept and the slope on the resulting straight line.

The results are as follows:

	$\mathscr{K}_1$	$\mathscr{K}_2$
Cu	1.5×10^{-2}	1.0×10^{-4}
Zn	6.0×10^{-2}	2.7×10^{-4}
Cd	7.5×10^{-2}	1.7×10^{-3}

3. The mechanism of reaction between Cu, Zn, Cd and γ-Al_2O_3 in seawater has been studied in detail. Since the ratio of exchange (1 percent)-pH graph is a "S shaped" curve, it is possible to deduce that the chemical reaction is the cation exchange. Since $pH_{range\ of\ ion\ exchange} = 4$, a monovalence cation exchange mechanism can be further deduced:



$$\equiv Al{-}OH + ML^+ \rightleftharpoons \equiv Al{-}O{-}ML + H^+$$

$$(M = Cu, Zn, Cd;\ L = Cl^-, OH^-)$$

(Paper received 26 Jul 87.)

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9717
CSO: 4009/1078

STUDY OF CRYSTALLIZATION PROCESS IN METALLIC GLASS $Zr_{63.2}Ni_{36.8}$

Hefei ZHONGGUO KEXUE JISHU DAXUE XUEBAO [JOURNAL OF CHINA UNIVERSITY OF SCIENCE AND TECHNOLOGY] in Chinese Vol 16 No 4, Dec 86 pp 391-395

[English abstract of article by Wei Qin [7279 2953] of the Institute of Solid State Physics, Chinese Academy of Sciences; Zhang Qirui [1728 0366 3843] of the University of Science and Technology of China]

[Text] By means of resistance, differential thermal capacity and X-ray diffraction measurements, the crystallization process of metallic glass $Zr_{63.2}Ni_{36.8}$ has been examined in the temperature region of 20-700°C. It has been found that the bottom-centered orthogonal NiZr is first precipitated at a temperature of 380°C, then the $NiZr_2$ with body-centered tetragonal structure at 425°C. Most of the material travels to $NiZr_2$, with a little to NiZr, at 550°C. (Paper received 10 Dec 85.)

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CSO: 4009/1052

STRUCTURAL CHARACTERISTICS IN LANTHANIDE COORDINATION COMPOUNDS

Hefei ZHONGGUO KEXUE JISHU DAXUE XUEBAO [JOURNAL OF CHINA UNIVERSITY OF SCIENCE AND TECHNOLOGY] in Chinese Vol 16 No 4, Dec 86 pp 455-460

[English abstract of article by Sun Pengnian [1327 7720 1628] of the Department of Biology, University of Science and Technology of China; Feng Xizhang [7458 6932 3864], et al., of the Institute of High Energy Physics]

[Text] Three steric parameters, i.e., solid angle factor (SAF), fan angle (FA) and coordination vector (r), are introduced to describe the steric packing around the center. In treatment of more than 160 structures of lanthanide coordination compounds, it was found that the sums of the ligand solid angle factors (SAS) are concentrated in a stable region, i.e., $\overline{SAS} = 0.78$, $\sigma = 0.05$. The Packing Saturation Rule provides distinct evidence that "coordination saturation" in lanthanide is actually the saturation in coordination space.

The Packing Center Rule reports that the vector sum of the ligand SAFs, i.e., $\Sigma SAF \cdot r$, of each structure closely approaches zero with an average $\Sigma SAF \cdot r = 0.02$ and a standard deviation of 0.015. The bond angles of coordination pattern MA_3B are easily obtained according to the second rule. The calculated results are in good agreement with the reported experimental results.

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CSO: 4009/1052

ELECTRONIC STRUCTURE OF $[Cu(C_7H_4NO_3S)_2(H_2O)_4]\cdot 2H_2O$ CRYSTAL

Hefei ZHONGGUO KEXUE JISHU DAXUE XUEBAO [JOURNAL OF CHINA UNIVERSITY OF SCIENCE AND TECHNOLOGY] in Chinese Vol 16 No 4, Dec 86 pp 407-411

[English abstract of article by Li Jianmin [2621 0256 3046], et al., of the Department of Modern Chemistry]

[Text] The electronic absorption spectrum and ESR spectrum of the crystal of the compound $[Cu(C_7H_4NO_3S)_2(H_2O)_4]\cdot 2H_2O$ are measured. The experimental results are discussed quantitatively by using the ligand field theory and the radial wave function of nonfree Cu(II). The electronic structure of this compound agrees with its crystal structure. (Paper received 26 Feb 86.)

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CSO: 4009/1052

INTERACTIONS IN POLY(ε-CAPROLACTONE)-BASED POLYURETHANE AND IN ITS BLENDS WITH POLY(VINYL CHLORIDE) BY FTIR

Hefei ZHONGGUO KEXUE JISHU DAXUE XUEBAO [JOURNAL OF CHINA UNIVERSITY OF SCIENCE AND TECHNOLOGY] in Chinese Vol 16 No 4, Dec 86 pp 402-406

[English abstract of article by Liu Yan [0491 1750], et al., of the Department of Applied Chemistry]

[Text] The annealing, quenching and different spectra have proved that in the amorphous states, hydrogen bonds exist between the carbonyl group of the soft segments and the NH bond of the hard segments. However, the crystallization leads to the formation of more hydrogen bonds between the hard segments because the blend system bands of the carbonyl and amide II groups shift to a lower frequency with the increasing of the poly(vinyl chloride).

The difference spectra indicates that polyurethane in blends with poly(vinyl chloride) still retains considerable order. (Paper received 19 Nov 85.)

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CSO: 4009/1052

ON FUZZY INTEGRATION

Hefei ZHONGGUO KEXUE JISHU DAXUE XUEBAO [JOURNAL OF CHINA UNIVERSITY OF SCIENCE AND TECHNOLOGY] in Chinese Vol 16 No 4, Dec 86 pp 461-466

[English abstract of article by He Chen [0149 3819] of the Department of Applied Mathematics, Shanghai Jiaotong University]

[Text] This paper adopts the concept of fuzzy measures proposed by E.P. Klement and shows that, if T is a continuous triangular norm, a T-fuzzy measure m is completely determined by a family of (positive) measures $\{\mu_\alpha:\alpha\ [0,1]\}$ satisfying the conditions (1.10)-(1.13), and μ_α and m are connected by (1.9). In view if this, it seems natural and reasonable to understand a fuzzy integral $\int_z fdm$ just as a function $\alpha\mapsto\int_z fd\mu_\alpha$, rather than as a single value as in the case of ordinary integrals. Based on this, the author tries to present the famous Riesz representation theorm in the form of fuzzy measures. (Paper received 29 Aug 85.)

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9717
CSO: 4009/1091

STUDY OF ANODIC OXIDE FILM-SEMICONDUCTOR INTERFACE OF $Hg_{1-x}Cd_xTe$

Shanghai HONGWAI YANJIU [CHINESE JOURNAL OF INFRARED RESEARCH] in Chinese
Vol 6 No 1, Feb 87 pp 21-27

[English abstract of article by Xu Zhenjia [6079 2182 0857], et al., of the Institute of Semiconductors, Chinese Academy of Sciences; Fang Jiaxiong [2455 3167 3574] of Shanghai Institute of Technical Physics, Chinese Academy of Sciences]

[Text] The composition and chemical states of the interface of $Hg_{0.8}Cd_{0.2}Te$ (MCT) with anodic oxide films of 35, 50, 55 and 65 nm are investigated with X-ray Photoelectron Spectroscopy (XPS). The results of the quantitative measurement of XPS show that the composition of the anodic oxide is 58~60 percent O, 22~26 percent Te, 10~13 percent Cd and 3~4 percent Hg.

The near-interface semiconductor side exhibits a region with 14~34 percent less Hg than in the bulk semiconductor. The mercury depletion width is dependent on the oxide thickness. (Paper received 15 Oct 85; revised 29 Mar 86.)

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9717
CSO: 4009/1093

IDENTIFICATION OF MODEL FOR DISTRIBUTION OF POTENTIAL AND CURRENT DENSITY ON ELECTRODE SURFACE

Tianjin TIANJIN DAXUE XUEBAO [JOURNAL OF TIANJIN UNIVERSITY] in Chinese No 1, Jan 87 pp 42-52

[English abstract of article by Shen Manli [3088 2581 7787], et al., of the Department of Applied Chemistry]

[Text] This paper describes a mathematical model for the distribution of potential and current density on the plates of lead-acid batteries. It can be used to predict important behaviors of the battery during discharging, such as cell voltage, the distribution of potential and current density, and the utilization of active materials on the plates. It also provides the theoretical basis for the optimization of battery design. (Paper received 18 Oct 85.)

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CSO: 4008/192

EXTRACTION OF URANIUM BY D_2EHPA EMULSION MEMBRANE

Tianjin TIANJIN DAXUE XUEBAO [JOURNAL OF TIANJIN UNIVERSITY] in Chinese No 1, Jan 87 pp 53-59

[English abstract of article by Wang Deyi [3769 1795 5030], et al., of the Department of Chemical Engineering]

[Text] This paper studies the preparation of the stable emulsion membrane containing D_2EHPA to extract uranium and the separation of uranium from iron. The results show that the best membrane should contain 2-4 percent span 80 and that the extraction efficiency of uranium and the separation coefficient of uranium can be very satisfactory when the membrane contains 8-18 percent D_2EHPA. (Paper received 2 Nov 85.)

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CSO: 4008/192

DYNAMIC RESPONSE OF FLOATING CRANE DERRICK TO WAVES

Tianjin TIANJIN DAXUE XUEBAO [JOURNAL OF TIANJIN UNIVERSITY] in Chinese No 1, Jan 87 pp 69-76

[English abstract of article by Dong Yanqiu [5516 5333 4428], et al., of the Department of Ocean and Naval Architecture Engineering]

[Text] Accidents involving fixed derricks of floating cranes often occur in seaways. According to practical requirements, the authors of this paper have studied the dynamic response of derricks under the action of waves and present a mechanical model and calculating method based on new knowledge of fluid mechanics and solid mechanics. This paper intends to find a proper way of considering the dynamic responses under the action of waves when flotation cranes are designed.

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CSO: 4008/192

RESEARCH ON SYNTHESIZING CHINESE SPEECH BY RULE IN TIME DOMAIN

Tianjin TIANJIN DAXUE XUEBAO [JOURNAL OF TIANJIN UNIVERSITY] in Chinese No 1, Jan 87 pp 101-108

[English abstract of article by Dang Jianwu of Tianjin University; Wu Wenhu [0702 2429 5706] of Qinghua University]

[Text] This paper researches the characteristics of the synthesis of Chinese speech in time domain and introduces the method to do this. The authors discuss how to synthesize Chinese speech which has four tones, analyzing and comparing different methods. They also discuss the relationship between the smoothness of transition among compound vowels (sometimes with a terminal n or ng) and the formant frequency, and analyze the effect of the transition sound on syllables of Chinese speech. (Paper received 23 Oct 85.)

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9717
CSO: 4008/1092

WELL-CONDITIONED SEARCH DIRECTION IN OPTIMIZATION TECHNIQUES AND ITS REALIZATION IN METHOD OF NONLINEAR LEAST SQUARES

Chongqing CHONGQING DAXUE XUEBAO [JOURNAL OF CHONGQING UNIVERSITY] in Chinese Vol 9 No 4, Dec 86 pp 27-35

[English abstract of article by Fu Li [0102 7765], et al., of the Department of Applied Mathematics]

[Text] The demands made on search direction in optimization techniques are explained from a practical point of view, and the idea of constructing the search direction to be as efficient as possible under the constraint of well-conditionedness is presented. A new iteration formula is derived that is different from the traditional methods for solving least squares problems to realize this idea. The numerical computations show that the method is more reliable and efficient than the traditional ones. (Paper received 20 Aug 86.)

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CSO: 4009/1089

APPLIED SCIENCES

CONCLUDING REPORT ON LIQUID SCINTILLATORS LSPB I, II, AND THEIR APPLICATIONS TO NUCLEAR MEDICINE

Chongqing CHONGQING DAXUE XUEBAO [JOURNAL OF CHONGQING UNIVERSITY] in Chinese Vol 9 No 4, Dec 86 pp 49-71

[English abstract of article by Yang Xueheng [2799 1331 1854], et al., of the Particle Physics Laboratory, Chongqing University; Huang Xiujun [7806 4423 0689], et al., of Chongqing Planned Parenthood Institute; Li Tianxing [2621 1131 2502], et al., of the Institute of Field Surgery, Third Military Medical College; Zhang Meiyuan [1728 5019 0061], et al., of Shanghai Endocrinology Institute]

[Text] Two new species of organic liquid scintillators, labeled LSPB I and II, have been supplied by the authors from Chongqing University. Their main technical indices agree with those of the scintillator, commonly used in China and abroad, which contains dimethylbenzene as the solvent. However, they have a higher flash point, lower volatility, good temperature effect and abundant resource and, in particular, are less poisonous. LSPB I and II are especially suitable for the measurement of low-energy β rays, such as are encountered in biology, medicine, agriculture, etc.

A report is given on the performance of LSPB I and II and their clinical practice during the past three years in nuclear medicine. Experiments show that they perform satisfactorily. The authors report that LSPB I and II are comparatively ideal scintillators to be used instead of the conventional ones.
(Paper received 12 Apr 86.)

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CSO: 4009/1089

DEPRESSED SCATTERING IN CRYSTAL Nd^{3+}:YAG

Chongqing CHONGQING DAXUE XUEBAO [JOURNAL OF CHONGQING UNIVERSITY] in Chinese Vol 9 No 4, Dec 86 pp 72-79

[English abstract of article by Ni Zhongxiang [0242 1350 4382] of the Department of Applied Physics]

[Text] The process of crystal growth in a resistance furnace is qualitatively discussed. It is found that the scattering particle in the melt will move away from the boundary particle and the capture probability of the scattering particle will decrease, if only we control forced convection $\vec{F}_f$ and natural convection $\vec{F}_n$ and keep $<\vec{F}_n, \vec{F}_f> <120°$ at a certain ratio of height and diameter. (Paper received 12 Apr 86.)

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CSO: 4009/1089

APPLIED SCIENCES

OPTICAL METHOD FOR STUDYING ELECTROCHEMICAL REACTION, OXIDATION AND REDUCTION OF SURFACE FILMS ON IRON

Chongqing CHONGQING DAXUE XUEBAO [JOURNAL OF CHONGQING UNIVERSITY] in Chinese Vol 9 No 4, Dec 86 pp 109-117

[English abstract of article by Huang Zongqing [7806 1350 0615] of the Department of Applied Chemistry]

[Text] The surface film formed on iron in alkaline solution has been studied by ellipsometry. The growth, dissolution and conversion of iron surface films are discussed regarding the optical and electrochemical parameters and the changing law of these parameters during the anodic and cathodic cycling of the iron surface films in alkaline electrolyte solutions. The presentation of soluble intermediates during the oxidation and reduction processes is also discussed. The experimental results indicate that the information provided by this optical method is useful for the observation and explanation of the experimental phenomena, and this cannot be done by the electrochemical method. The optical method will be a very useful tool for studying electrochemical reactions in the future. (Paper received 1 Apr 86.)

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CSO: 4009/1089

ELLIPSOMETRIC STUDY OF ANODIC OXIDE FILMS ON TITANIUM IN AMMONIUM SULPHATE SOLUTION

Chongqing CHONGQING DAXUE XUEBAO [JOURNAL OF CHONGQING UNIVERSITY] in Chinese Vol 9 No 4, Dec 86 pp 118-125

[English abstract of article by Xie Shangfen [6200 0006 5358], et al., of the Department of Applied Chemistry]

[Text] The anodic oxide film galvanostatically formed on titanium has been studied by ellipsometry, TEM and XPS analysis. The growth, composition and breakdown of the films at 0-20 v (SCE) are discussed. Experimental results support the single-layer model. The film growth rate is 2.65, 2.5, 2.5, 2.2 and 2.0 nm/v in 0.02, 0.05, 0.1, 1 and 2.5 mA/cm^2 respectively. The authors suggest that the film composition is $TiO_2(H_2O)_{0.9}$, the film density is 3.19 g/cm^3, and the breakdown potential is proportional to the logarithm of current density. (Paper received 1 Apr 86.)

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COLOR AND COLOR CHANGES OF SURFACE OXIDE FILMS ON TITANIUM

Chongqing CHONGQING DAXUE XUEBAO [JOURNAL OF CHONGQING UNIVERSITY] in Chinese
Vol 9 No 4, Dec 86 pp 126-133

[English abstract of article by Tong Jiajie [4547 1367 2638], et al., of the Department of Applied Chemistry]

[Text] Linear sweep voltametry has been used to form varieties of anodic oxide films with colors on titanium. The electrochromic behaviors of the anodic oxide films on titanium in aqueous solutions have been studied using the same method. The ellipsometry and XPS analysis have also been used to study the relationships among the colors, thickness, components of the anodic oxide films on titanium and the applied potential. A process involving the injection or extraction of electrons and ions is suggested to explain the overall electrochromism of the anodic oxide films on titanium. (Paper received 31 Mar 86.)

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COMPUTER AIDED CODING SOFTWARE SYSTEM, CD-CAC-1

Chongqing CHONGQING DAXUE XUEBAO [JOURNAL OF CHONGQING UNIVERSITY] in Chinese Vol 9 No 1, Mar 86 pp 11-18

[English abstract of article by Li Nan [2621 0589], et al., of the Department of Mechanical Engineering]

[Text] A software system, CD-CAC-1, for the microcomputer (IBM-PC/XT) is described. The system is designed to perform the following functions: coding, revising, counting and typing output according to some definite formats. The screen display and output are all in Chinese. Some new ideas and methods have been adopted to improve and optimize the program. Test results have been successful. Developing such a software system is the primary job of Computer Aided GT. (Paper received 3 Jun 85.)

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CSO: 4009/1087

CUSAS-84: STRUCTURAL ANALYSIS PROGRAM SYSTEM ON MICROCOMPUTER

Chongqing CHONGQING DAXUE XUEBAO [JOURNAL OF CHONGQING UNIVERSITY] in Chinese Vol (No 1, Mar 86 pp 19-24

[English abstract of article by Zhang Ruqing [1728 3067 3237], et al., of the Institute of Engineering Mechanics]

[Text] The general development of structural analysis programs in recent years is discussed. The importance of using microcomputers in structural analysis is emphasized. The characteristics of using microcomputers for structural analysis are also discussed with respect to program structure, data management, choice of methods, making full use of computer resources, etc. A program system of static and dynamic analysis for various structures is developed for the microcomputer and is called CUSAS-84. (Paper received 8 May 85.)

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CSO: 4009/1087

DATA STRUCTURES FOR PRACTICAL INTELLIGENT SYSTEM

Chongqing CHONGQING DAXUE XUEBAO [JOURNAL OF CHONGQING UNIVERSITY] in Chinese Vol 9 No 1, Mar 86 pp 25-30

[English abstract of article by Xiao Baolin [5135 1405 7792] of the Department of Computers and Automation]

[Text] An expert system on computer for diagnosis and treatment of Gong Zixian's pyelonephritis based on an overall analysis of the illness and the patient's condition is presented. All types of data structures used in the program are analyzed in detail. (Paper received 26 Feb 85.)

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9717
CSO: 4009/1087

ERROR PERFORMANCE IMPROVEMENT FOR ANGLE MODULATION SYSTEMS IN PRESENCE OF CO-CHANNEL OR MULTIPATH INTERFERENCE USING DIVERSITY

Beijing TONGXIN XUEBAO [JOURNAL OF CHINA INSTITUTE OF COMMUNICATIONS] in Chinese Vol 7 No 1, Jan 86 pp 10-20

[English abstract of article by Wu Kerang [0114 0344 6245] of Northwest Telecommunication Engineering Institute; N. Morinaga and T. Namekawa of Osaka University]

[Text] The error rate performance improvement of angle modulation systems involving digital FM, MSK and DPSK using the differential post-detection diversity combining technique has been studied in the presence of co-channel or multipath interference. The improvement of the error rate performance is presented with a simple closed form of the system parameters by using the residue theorem. The authors' results show that the diversity technique is quite effective not only on the envelope fading of received signals, but on the co-channel/multipath interference and random FM as well. In particular, the error probability due to random FM using L-diversity combining performs almost the same as does the L-produce-law of that for non-diversity. The results also show that diversity cannot reduce bit error when a desired D-wave or undesired U-wave arrive with relatively longer delay time and almost equal levels. An additional technique (e.g., adaptive equalizer and error-correcting codes) is required in order to realize high-quality transmission performance. (Paper received 10 Nov 84.)

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CSO: 4009/186

NEW DESIGN FOR HIGHLY EFFICIENT CASSEGRAINIAN ANTENNA

Beijing TONGXIN XUEBAO [JOURNAL OF CHINA INSTITUTE OF COMMUNICATIONS] in Chinese Vol 7 No 1, Jan 86 pp 31-35

[English abstract of article by Mao Yukuan [5403 0060 1401] of Northwest Telecommunication Engineering Institute]

[Text] A scalar feed and shaped reflectors are the conditions needed to make a Cassegrainian antenna more efficient. Shaping reflectors using geometrical optics is the most commonly used method now. The diffraction theory for shaping proposed by P.T. Wood can bring higher efficiency than the geometrical optics designs. Efficiency can also be improved by shaping only the subreflector in medium and small antennas ($D \leq 100\lambda$). All these methods for achieving higher efficiency are based on improvement of the aperture illumination. A method for improving efficiency by reducing the diffraction loss in a Cassegrainian antenna is proposed in this paper, however the case of subreflector shaping is only considered. The general principle outlined here can also be applied to main and subreflector shaping with more complicated calculation and manufacturing. The theoretically calculated efficiencies and feed-subreflector radiation polar patterns relating to (1) a typical Cassegrainian antenna, (2) a typical Cassegrainian antenna with an extended subreflector, and (3) a paraboloid main reflector with an extended subreflector shaped by the diffraction theory are compared and the frequency characteristics of the latter are computed. (Paper received 28 Jun 84.)

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CSO: 4009/186

SLOW FREQUENCY HOPPING (FH) SYNCHRONIZATION METHOD AND EXPERIMENTAL SIMULATION

Beijing TONGXIN XUEBAO [JOURNAL OF CHINA INSTITUTE OF COMMUNICATIONS]
in Chinese Vol 7 No 1, Jan 86 pp 36-43

[English abstract of article by Xiang Haige [7309 3189 2706], et al., of Beijing University]

[Text] A synchronization method of slow Frequency-Hopping/Binary Frequency Shift Key (FH/2FSK) communication systems is discussed in this paper. The acquisition and tracking of the information bit synchronization and of frequency-hopping pattern synchronization are shown. The analysis of the synchronization performance and the use of a microprocessor to control the synchronization operation are described in detail. a FH communication synchronization experimental simulation system with microprocessors is given. Some results of digital voice transmission and FH pattern synchronization are obtained which show that the synchronization scheme is practicable. (Paper received 19 Mar 84.)

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DESIGN OPTIMIZATION FOR SINGLE-ENDED FLYBACK CONVERTER

Beijing TONGXIN XUEBAO [JOURNAL OF CHINA INSTITUTE OF COMMUNICATIONS] in Chinese Vol 7 No 1, Jan 86 pp 52-59

[English abstract of article by Cai Xuansan [5591 1357 0005], et al., of Qinghua University]

[Text] In this paper, the optimum design is employed for a single-ended flyback converter with four outputs. The mathematical model contains 16 variables and 16 inequalities as constraints to minimize the weighted sum of the power loss and weight of the converter. The Augmented Lagrangian Multiplier Penalty Function Technique is adopted in forming the CAD program with FORTRAN-IV. By treating the switching frequency as a constant in each computer run, a set os suboptimum design solutions can be obtained by varying the frequency from 20 to 60 kHz in 10 kHz steps. The U-shaped curve can be observed by plotting the total loss characteristic against frequency. The following optimum design results are also obtained: (1) The optimum dc duty ratio is about 0.3-0.34. (2) The converter should work at the critical state between the discontinuous and continuous state. (3) The air gap of the transformer corer should be large enough (e.g., 1.2-1.3 mm), etc. (Paper received 18 Apr 85.)

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CSO: 4009/1086

APPLIED SCIENCES

STUDY OF α-PARTICLE EMISSION MECHANISM IN N+Ni REACTION AT 96MeV

Beijing YUANZIHE WULI [CHINESE JOURNAL OF NUCLEAR PHYSICS] in Chinese Vol 8 No 2, May 86 pp 102-108

[English abstract of article by Fan Guoying [5400 0948 5391], et al., of the Institute of Modern Physics, Chinese Academy of Sciences, Lanzhou]

[Text] Energy and angular correlations of α-particles in coincidence with light fragments from ^{14}N+Ni reaction at 96 MeV are measured. The coincident spectra of α-particles indicate the sequential decay pattern of the heavy fragments. Near the direction of the detected light fragments, the shadow effect is observed.

CSO: 4009/37

STUDY OF ELECTRIC QUADRUPOLE INTERACTION OF $^{112}Sn(6^+)$ ISOMERS AND RADIATION DAMAGE IN HEXAGONAL Cd

Beijing YUANZIHE WULI [CHINESE JOURNAL OF NUCLEAR PHYSICS] in Chinese Vol 8 No 2, May 86 pp 109-112, 119

[English abstract of article by Zhu Shengyun [2612 0581 0061], et al., of the Institute of Atomic Energy, Beijing]

[Text] Quadrupole coupling constants of the $^{112}Sn(6^+)$ isomers implanted into Cd are measured with the time differential perturbed angular distribution method from 290K to 500K. Radiation damage in Cd is studied. The experiment shows that the temperature dependence of the quadrupole coupling constants of the $^{112}Sn(6^+)$ isomer in Cd follows the $T^{3/2}$ law: $\nu_Q(T) = \nu_Q(0)(1-B\cdot T^{3/2})$ with the slope $B=1.97(31)\cdot10^{-5}K^{-3/2}$. The self-diffusion model of point defect is used to fit the measured temperature dependence of the effective nuclear alignment. The migration energy of the induced defects in Cd is found to be 0.44 eV, which is in accordance with the migration energy of monovacancy in Cd. The fraction of ^{112}Sn nuclei which trap defects at low temperatures is 49.4 percent.

CSO: 4009/37

MEASUREMENT OF LIFETIME OF ^{28}Si LEVEL

Beijing YUANZIHE WULI [CHINESE JOURNAL OF NUCLEAR PHYSICS] in Chinese Vol 8 No 2, May 86 pp 113-119

[English abstract of article by Li Shenggang [2621 3932 1511], et al., of the Institute of Atomic Energy, Beijing]

[Text] The lifetimes of levels of ^{28}Si for 4.62, 6.89, 7.42, 7.80, 8.54, 8.94 and 10.4 MeV are measured by the ^{27}Al(p,γ)^{28}Si reaction and the Doppler shift attenuation method on the 2.5 MeV Van de Graaff accelerator at the Institute of Atomic Energy. According to Blaugrundd's theory, the programs for calculating the lifetimes of levels of the nucleus are made. The lifetimes of the energy levels are calculated by the numeral integral method. The results are quite satisfactory.

CSO: 4009/37

FINE STRUCTURE OF NUCLEAR CHARGE RADIUS

Beijing YUANZIHE WULI [CHINESE JOURNAL OF NUCLEAR PHYSICS] in Chinese Vol 8 No 2, May 86 pp 132-139

[English abstract of article by Pan Zhengying [3382 2973 3841], et al., of Fudan University, Shanghai]

[Text] A new nuclear charge radius formula is developed which incorporates contributions from the size and shape of the system as well as from the diffuseness of the surface. A new asymmetry term is included to account for the nuclear charge radii of nuclides far from β-stability. A comparison of predictions of several main radius expressions with experiments for 332 nuclides are given and the resulting rms deviations are tabulated.

CSO: 4009/37

EFFECTS OF QUADRUPOLE VIBRATION OF FRAGMENTS ON μ^- FINAL-STATE PROBABILITIES

Beijing YUANZIHE WULI [CHINESE JOURNAL OF NUCLEAR PHYSICS] in Chinese Vol 8 No 2, May 86 pp 140-146, 153

[English abstract of article by Zheng Guotong [6774 0948 2717] of Hangzhou Teacher's College; Wang Yansen [3769 3508 2773], et al., of Fudan University, Shanghai]

[Text] The muon final-state probabilities after muon-induced fission of ^{238}U are calculated by using the LCAO (Linear Combination of Atomic Orbital) method. The ordinary viscosity of the fission nucleus, the deformations and quadrupole vibrations of two fragments are taken into account. The calculated results are compared with those obtained by neglecting the quadrupole vibrations.

CSO: 4009/37

DESIGN OF DUAL-MODE GUN FOR HIGH CURRENT ELECTRON LINEAR ACCELERATOR

Beijing YUANZIHE WULI [CHINESE JOURNAL OF NUCLEAR PHYSICS] in Chinese Vol 8 No 2, May 86 pp 160-164, 178

[English abstract of article by Wang Houwen [3769 0624 4489] of Nanjing University]

[Text] A dual mode electron gun with two grids is described in this paper. By adjusting voltages of two grids and focusing the electrode, the gun can produce an electron beam with good laminarity under two operating conditions of a high current electron linear accelerator.

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BRIEFS

ADVANCED COMPUTERS IN OIL EXPLORATION--Beijing, 27 Feb (XINHUA)--Advanced computers are being used to prospect for oil in the world's second-largest desert, the Taklimakan, in northwestern China, today's PEOPLE'S DAILY reported. The "Yinhe," a supercomputer with a capacity of 100 million operations per second, has been used to process geological data since 1982. China joined forces with the United States to survey the geology of the Taklimakan Desert in 1983 and the Sino-U.S. prospecting team has crossed the desert 22 times. They have found a number of oil-gas textures, and even water resources. Now, China is prospecting for oil in every corner of its territory, except the Tibet Autonomous Region, the paper added. [Text] [Beijing XINHUA in English 1611 GMT 27 Feb 87] /8309

RENMIN RIBAO ON MICROCIRCULATION RESEARCH WORK--Beijing, 2 Mar (XINHUA)--Chinese scientists have produced an advanced multi-system instrument for detecting abnormal blood flow, which probes inside the smallest veins and capillaries in the body, today's PEOPLE'S DAILY reported. The instrument, developed by scientists of the Research Institute of Microcirculation under the Chinese Academy of Medical Sciences, can be used for animal research, clinical examinations and experimental research on the human body and in microcirculatory research, according to the report. The instrument consists of a life-support system, microscopic closed-circuit television system, a biological information gathering and analysing system and an electronic data-processing system, the report said. Xiu Ruijuan, director of the research institute, sponsored the development of the microcirculation research instrument over the past two years, the report said. [Text] [Beijing XINHUA in English 0841 GMT 2 Mar 87] /8309

CSO: 4010/1019

CHEMICAL SYNTHESIS OF NEW CYTOTOXIC PEPTIDE

Shanghai SHENGWUHUAXUE YU SHENGWUWULI XUEBAO [ACTA BIOCHEMICA ET BIOPHYSICA SINICA] in Chinese Vol 18, No 1, Jan 86 pp 60-73

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[Text] Abstract: This paper describes the synthesis of a cytotoxic peptide with bacteria-inhibiting activity. The sequence of its 60 amino acids is identical to that of MT-D_1, a newly discovered cytotoxin from Chinese cobra venom, except that the order of the 48th and 49th amino acid residues, Leu and Val, are reversed. The total synthesis is accomplished through the preparation of 13 peptide fragments by literature methods, which are then condensed on resin support sequentially. The overall yield of these condensations approaches 60 percent. The product, after HF treatment, column chromatographic separation, oxidation, and further purification, showed the same biological activity and antigenicity as those of the natural MT-D_1.

We have reported the isolation of five membrane toxin components (MT-A, MT-B, MT-C, MT-D_2, and MT-D_1)* from the native Guangdong cobra by equilibrium chromatography on a sulfone-type dextran gel.[1] The first four components are identical to the Taiwanese cobra cardiotoxins CTX-I, CTX-II, CTX-III, and CTX-IV, whose primary structures are known.[2,3] MT-D_1, which contains 60

*Abbreviations: BOC, t-butyloxycarbonyl; BPOC, biphenylisopropyloxycarbonyl; BTA, 3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine; BZL, benzyl; CHA, cyclohexylamine; DCCI, N,N'-dicyclohexylcarbodiimide; DCB, 2,6-dichlorobenzyl; DMB, 3,4-dimethylbenzyl; DMF, dimethylformamide; DMSO, dimethylsulfoxide; E, diethyl ether; EA, ethyl acetate; ϕ, resin; IBCF, isobutylchloroformate; MA, mixed anhydrides; NMM, N-methylmorpholine; -ONP, p-nitrophenyl ester; -OSu, N-hydroxysuccinimide ester; PE, petroleum ether; TLC, thin layer chromatography on silica gel; TFA, trifluoroacetic acid; THF, tetrahydrofuran; Z, benzyloxycarbonyl; ZCL, 2-chlorobenzyloxycarbonyl. The single-letter notations of amino acids and residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp.

amino acid residues, is the only new toxin.[4] Like cytotoxins from other sources,[5] the Chinese cobra toxins can cause the depolarization of excitable membrane, contraction of skeletal muscles, systolic cardiac arrest, and direct cytolysis of Yoshida sarcoma cells.[6] We also observed their destruction of cabbage chloroplasts[7] and their effect on the inner membrane calcium channel that subsequently caused inhibition of the respiration of rat liver mitochondria.[8] Though there is a controversy over their mechanism of action, it is reasonable to call them membrane toxins or membrane-active polypeptides.

Among the various activities of membrane toxins, the inhibition of E. coli growth is interesting because the diameter of the inhibition zone on an agar plate is proportional to the logarithmic concentration of the toxin. It was pointed out in the previous paper[4] that this property is parallel to its lysis activity of Yoshida sarcoma cell. Therefore, one can quickly and easily compare the structure-activity relationship of the cytotoxins based on the diameter of the inhibition zone. Among the above-mentioned five components, MT-D_1 is a highly potent cytotoxin, whose potency is about the same as that of MT-C.

Based on the primary structure of D_1 that has been elucidated,[10] the amino acid sequence homology among the components of Chinese cobra cytotoxin and its Indian counterparts can be easily seen. From Table 1, one can see that these types of molecules are highly conserved. With the exception of (Asn, Ser) and (Leu, Ala) interchange at positions 45, 47, and other scattered interchanges of similar residues such as (Leu, Val), (Tyr, Phe), and (Ala, Phe), the majority are not altered. It is readily noticeable that there exists an obvious variable region in the middle section of the molecule from positions 28 to 31. It has already been pointed out[6] that the toxicity of the MT's seems to be dependent on the presence or absence of a Lys residue in the region. Therefore, it would be very significant if one can chemically synthesize a membrane toxin based on the features of MT-D_1 (variable region: SNKM) and compare its biological activity. This article describes our attempts to carry out solid-phase condensations of protected peptide fragments,[1] which are prepared by literature methods, to form a new cytotoxin that is based on the sequence of MT-D_1 except that the Leu and Val at positions 48 and 49 are reversed.

To synthesize a pure 60mer peptide is still not an easy task today. D_1 especially contains nearly 40 percent of hydrophobic residues as well as eight cycteine and three methionine residues. These certainly will cause problems in the synthesis itself and subsequent treatments. Based on our experience in synthesizing longer polypeptides by fragment condensations[11-18] and taking into consideration the pore size limitations of cross-linked polystyrene resin, we adopted in this paper the approach of first synthesizing 13 smaller peptide fragments (tri- to hexa-peptides) by literature methods, followed by step-wise condensations with a resin-bound fragment (see Figure 1).

Table 1. Primary Structure and Variable Region of MT-D_1

	10	20
MT-D_1	LeuLysCysAsnLysLeuValProLeuPheTyrLysThrCysProAlaGlyLysAsnLeu	
MT-B	LeuLysCysAsnLysLeuValProLeuPheTyrLysThrCysProAlaGlyLysAsnLeu	
CT-I_2	LeuLysCysAsnLysLeuValProLeuPheTyrLysThrCysProAlaGlyLysAsnLeu	
	30	40
MT-D_1	CysTyrLysMetPheMetValSerAsnLysMetValProValLysArgGlyCysIleAsp	
MT-B	CysTyrLysMetPheMetValSerAsnLeuThrValProValLysArgGlyCysIleAsp	
CT-I_2	CysTyrLysMetTyrMetValAlaThrProLysValProValLysArgGlyCysIleAsp	
	50	60
MT-D_1	ValCysProLysSerSerLeuLeuValLysTyrValCysCysAsnThrAspArgCysAsn	
MT-B	ValCysProLysAsnSerAlaLeuValLysTyrValCysCysAsnThrAspArgCysAsn	
CT-I_2	ValCysProLysSerSerLeuValLeuLysTyrValCysCysAsnThrAspArgCysAsn	

Note: MT-B is equivalent to the Taiwanese cobra cytotoxin CTX-II and CT-I_2 to the Indian cobra cytotoxin 2. Positions 28-31 constitute the middle section variable region and positions 45-48 the C-terminal variable region.

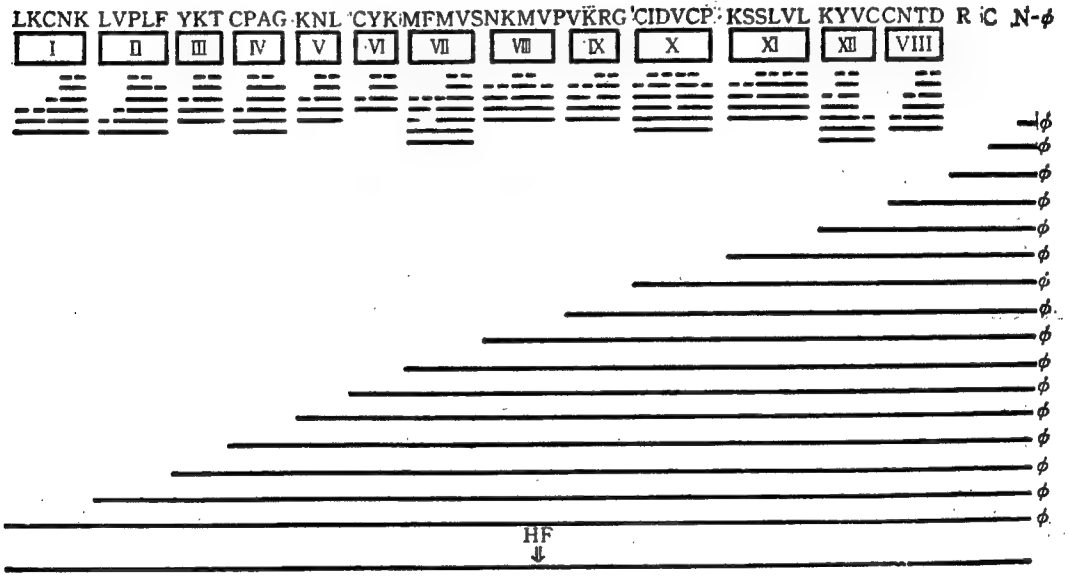

Figure 1. Synthetic Scheme of Cytotoxin

[ ] denotes fragments by literature methods; see experimental section for detail.

Using smaller fragments inevitably increases the number of solid-phase condensations. Therefore, side chain protecting groups should be the more resistant ones. In this paper, we used ZCL or Z for the ϵ-amino group, -OBZL for the β-carboxyl group, -BZL for the aliphatic hydroxyl and sulfhydryl group, -DCB for phenol, and -TOS for the guanidino group. The less stable Z was used only in the final few rounds of solid-phase coupling. The α-amino groups were generally protected with BOC. But with the elongation of the

peptide chain on the resin, more stringent conditions were required for the complete removal of BOC. Therefore, BPOC was chosen as a protecting group for the fragments used in the final rounds. For their complete removal, a higher HCl concentration and a longer reaction time are required, which certainly would have an adverse effect on sulfur-containing groups. Therefore, the presence of excess 1,2-ethanedithiol is necessary in acidic deprotections in order to prevent the oxidative destruction of Met and Cys.[14]

Data on the liquid-phase synthesis of protected peptides and their intermediates are described in the experimental section. Table 2 lists the physical data of the final 13 protected peptide fragments. They are all homogeneous as determined by amino acid composition analysis, elemental analysis, and TLC. It should be pointed out that it is more difficult for some strongly hydrophobic peptide fragments to form crystals in free acid form, so only the physical properties of their esters (e.g., peptide II) or CHA salts (e.g., peptides III, IV, VI, and VII) are listed. They were either acidified or saponified before their use in solid-phase condensation.

Solid-phase synthesis was carried out according to the flow chart in Figure 1. BOC-Asn-ϕ was prepared by reacting BOC-Asn-OH with 1 percent cross-linked chloromethylpolystyrene resin through the dimethylthioether intermediate.[15] After the removal of the BOC group, it was extended step-wise to form a tripeptide resin, BOC-ArgTOS-Cys(BZL)-Asn-ϕ. This resin was then condensed in order through normal procedure with the 13 protected peptide fragments, from peptide XIII to peptide I, to form the 60mer: Z-Leu-Lys(Z)-Cys(DMB)-Asn-Lys(Z)-Leu-Val-Pro-Leu-Phe-Tyr(BZL)-Lys(Z)-Thr(BZL)-Cys(BZL)-Pro-Ala-Gly-Lys(ZCL)-Asn-Leu-Cys(BZL)-Tyr(DCB)-Lys(ZCL)-Met-Phe-Met-Val-Ser(BZL)-Asn-Lys(ZCL)-Met-Val-Pro-Val-Lys(ZCL)-Arg(TOS)-Gly-Cys(BZL)-Ile-Asp(OBZL)-Val-Cys(BZL)-Pro-Lys(ZCL)-Ser(BZL)-Ser(BZL)-Leu-Val-Leu-Lys(ZCL)-Tyr(DCB)-Val-Cys(BZL)-Cys(BZL)-Asn-Thr(BZL)-Asp(OBZL)-Arg(TOS)-Cys(BZL)-Asn-ϕ.

The tripeptide resin starting material (290 mg, 0.55 mmol/g of the free amino group) was neutralized with triethylamine and mixed with a DMF or DMSO solution of the protected peptide. One equivalent of DCCI and the small amount of BTA were then added. After reacting for a certain period of time, the resin was washed and the unreacted amino group determined.[16] When the yield of a condensation was below 90 percent (e.g., peptide V), it was repeated with a reduced amount. Because of some adsorption of salicylaldehyde on the resin, this kind of experimental error tends to give a higher value to the unreacted amino group. After the completion of all condensations, the resin weighed 1.01 grams, three and a half times its original weight. The overall yield is 59 percent and the average step-wise yield was 96.1 percent as calculated from weight gain after making adjustment for the amount of resin consumed in each step for the amino group assay.

Table 2. Data on Solution Synthesis of Peptide Fragments

(1) 肽代号	(2) 肽段	熔点 m.p. °C	薄板层析 tlc. R_f*	(3) 比旋** $[\alpha]_D^{25}$	(4) 元素分析*** C	H	N	(5) 氨基酸组成分析
I	Z DMB Z Z-LeuLysCysAsnLys—OH	214	0.70	−15	61.36/61.70	7.07/7.01	10.22/9.93	Lys 1.82; Asp 1.05; Leu 1.02
II	Bpoc-LeuValProLeuPhe-OMe -OH	107～109 120～122	0.78 0.64	−88.0	68.92/68.62	7.76/7.79	8.54/8.33	Val 1.00; Phe 1.03; Leu 1.82; Pro 1.08
III	Bzl Z Bzl Bpoc-TyrLysThr-OH·CHA	138～140	0.78	+14.5				Thr 1.00; Lys 1.03; Tyr 0.73
IV	Bzl Bpoc—CysProAlaGly-OH	56～58	0.54	−50.5	63.72/64.07	6.23/6.27		Pro 0.95; Ala 1.00; Gly 1.02
V	Zcl Bpoc-LysAsnLeu-OH·H_2O	117～119	0.71	−20.5	59.74/60.10	6.38/6.68	8.73/8.76	Lys 1.18; Asn 0.92; Leu 1.00
VI	Bzl Dcb Zcl Bpoc-CysTyrLys-OH·CHA·MeOH	128～130	0.72		62.72/63.07	6.10/6.20	6.03/5.83	Lys 1.00; Tyr 0.82
VII	Bzl Bpoc-MetPheMetValSer-OH·CHA	168～170	0.83	−20.5	64.10/64.58	7.48/7.35	7.81/8.07	Phe 1.05; Met 2.02; Val 10.94; Ser 0.81
VIII	Zcl Boc-AsnLysMetValPro-OH	116～118	0.62	−65.0	53.27/53.29	6.75/6.82		Asp 1.03; Val 0.93; Lys 1.00; Pro 0.96; Met 0.82
IX	Zcl Tos Boc-ValLysArgGly-OH	134～138	0.60	−20.5	52.8/53.20	6.30/6.41	12.40/12.70	Lys 1.04; Arg 0.99; Gly 0.95; Val 1.00
X	Bzl oBz Bzl Boc—CysIleAspValCysPro-OH	192	0.74	−81.0	61.90/62.25	6.88/7.03	8.36/8.37	Ile 1.00; Val 0.94; Pro 1.08; Asp 1.00
XI	ZclBzl Bzl Boc-LysSerSerLeuValLeu-OH	132～133	0.82	−29.5	59.29/59.28	9.45/9.36	7.82/8.13	Lys 1.17; Val 1.05; Ser 1.70; Leu 2.00
XII	Zcl Dcb Bzl Boc-LysTyrValCys-OH	112～114	0.77	−14.0	58.59/58.70	6.04/5.98	6.57/6.71	Lys 1.00; Val 1.05; Tyr 0.86
XIII	Bzl Bzl oBz Boc-CysAsnThrAsp-OH	158～160	0.67	+6.0	60.02/59.91	6.23/6.25	8.37/8.52	Asp 1.92; Thr 1.12; Cys 0.96

*TLC developing system: the 37 system; see experimental section. **All are in MeOH, C=1 except for peptide I, which is in AcOH (C=0.5). ***Found/calculated. Phenol was added during hydrolysis for the amino acid analysis and cysteine residues were destroyed and not determined.

Key:

1. Peptide code
2. Peptide fragment
3. Optical rotation
4. Elemental analysis
5. Amino acid composition analysis

Table 3 summarizes the assay data obtained throughout the process. Because the weight of resin keeps increasing, the value of the free amino group per gram of resin drops accordingly. The amount of the exposed amino group determined for each step is very close to the calculated value, indicating that capping was not serious during the synthesis.

Table 3. Efficiency of Fragment Condensation on Resin

(1) 氨基成分 (树脂肽)	(2) 羧基成分 (肽片段)	(3) 缩合条件 (4) 肽片段过量倍	(5) 溶剂	30°C (6) 时间	(7) 残余氨基	(8) 氨基暴露量 (9) 实测	(10) 计算	(11) 缩合率	(12) 树脂肽重 (13) 实称	(14) 增重** 累计**	(15) 说 明
TOS Bzl H.ArgCysAsnφ	(XIII)	3.11	DMF*	hr 20	mmol/g 0.015	0.364	0.364	95%	mg 411	130	(16) 起始树脂为: 291 mg, 0.547 μmol/mg
H(54-60)-φ	(XII)	0.63	DMF	17	0.003	0.267	0.272	99%	527	236	
H(50-60)-φ	(XI)	0.67	DMSO	17	0.016	0.179	0.214	93%	638	348	
H(44-60)-φ	(X)	1.80	DMSO	44	0.014	0.174	0.178	92%	705	414	
H(38-60)-φ	(IX)	1.98	DMSO	44	0.005	0.140	0.156	96%	794	503	
H(34-60)-φ	(VIII)	1.74	DMSO	41	0.010	0.145	0.139	93%	827	536	
H(29-60)-φ	(VII)	1.00	DMSO	40	0.008	0.107	0.126	93%	—	—	
H(24-60)-φ	(VI)	1.00	DMSO	44	0.008	0.108	0.113	93%	905	614	
H(21-60)-φ	(V)	1.51 0.68	DMSO	40 68	0.006	0.110	0.107	95%	—	—	
H(18-60)-φ	(IV)	2.50	DMSO	44	0.005	0.105	0.102	95%	915	624	
H(14-60)-φ	(III)	1.28	DMSO	44	0.007	0.096	0.093	93%	—	—	
H(11-60)-φ	(II)	3.55	DMSO	41	0.007	0.094	0.088	93%	980	689	
H(6-60)-φ	(I)	2.26	DMSO	62	0.003	—	—	97%	1010	719	总增重347%。平均每步缩合率 96.1% (17)

*Condensation reagents: DCCI+BTA. The reaction was incomplete during the condensation of peptide V and was repeated.

**The amounts consumed for assays were ignored (about 20 mg for each step) so the actual weight gain should be greater.

Key:

1. Amino-end component (resin-bound peptide)
2. Carboxyl-end component (peptide fragment)
3. Condensation condition
4. Peptide fragment excess
5. Solvent
6. Time
7. Residual amino group
8. Exposed amino group
9. Found
10. Calculated
11. Condensation yield
12. Weight of peptide resin
13. Actual weight
14. Cumulative weight gain
15. Remark
16. Initial resin weight
17. Total weight gain 347 percent; average step-wise coupling yield 96.1 percent

It is well known[17] that, besides methionine, the sulfur atom on the protected cysteine residue can also be oxidized. The peptide-containing resin was reduced for 48 hours with 12.5 percent thiophenol/DMF for the reason that the above synthesis involved a large number of cysteine and even with excess 1,2-ethanedithiol added at each acid treatment, the possibility of sulfone formation could not be completely ruled out.

The 60mer resin (190 mg) was reacted, in the presence of p-cresol and a small amount of Met, with anhydrous liquid HF (5 ml) at 4°C for 2 hours, then 20°C for 15 minutes. After removal of HF by nitrogen, the residue was washed with anhydrous ether and dried under a stream of nitrogen. It was suspended in Tris-EDTA buffer containing 6M guanidine chloride (pH 8.3), reduced and extracted with excess mercaptoethanol (37°C). The supernatant from centrifugation was separated, under anaerobic conditions, by a Biogel P_6 column (1.5x57 cm) in 0.1N HOAc (see Figure 2). The high polymers (peak 1) and small molecules (peak 3) were discarded and peak 2, whose elution position corresponds to that of the natural toxin D_1, was collected under nitrogen and freeze-dried immediately to get 62.5 mg of the material. The overall yield of HF treatment and initial purification is 73 percent.

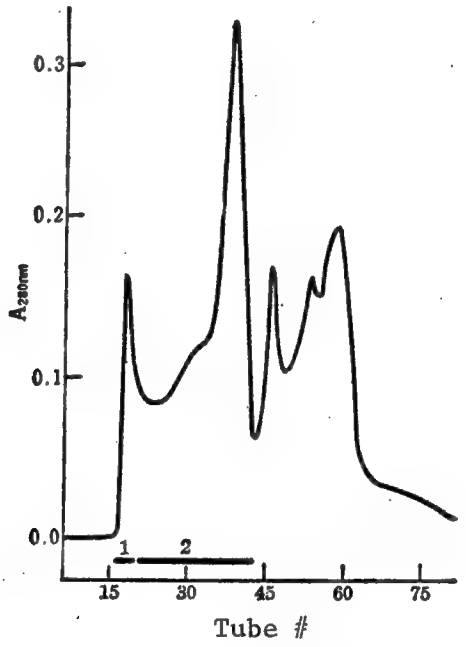

Figure 2. Gel Filtration of Crude Product on Column of Biogel P_6

Eluant: 0.1N HOAc (under N_2). Volume collected in each tube: 2 ml. Peak 1: unretained portion; peak 2: reduced product (collected under N_2); the latter portion of peak 2 are salts as determined by conductivity.

The above freeze-dried powder was dissolved in 1,150 ml of oxidation solution to make the final concentration as follows: phosphate buffer (pH 7.08), 0.026M; protein, 54 ug/ml; and NaN_3, 0.174 percent. After oxidation at 25°C for 10 days, the solution was centrifuged to remove traces of precipitate, and the supernatant, after concentration under reduced pressure, was put in a dialysis bag (MW cut-off: 2,000) and dialyzed against 1N HOAc to remove most of the salts. Figure 3 shows the gel permeation chromatogram of the oxidized product on a column of Sephadex G-15 (2.2x83 cm). The eluants were collected in three portions according to the absorption peaks and freeze-dried to get: peak I 9.7 mg, peak II 5.5 mg, and peak III 5.0 mg. By their retention, it can be seen that peak I is apparently the cross-linked product that is not retained and peak III has the same elution time as that of the natural toxin D_1. As determined by the electrophoresis on a cellulose acetate membrane at pH 6.0 (see Figure 4A), peak I basically does not migrate, peak II migrates slightly slower than the natural product, and the major portion of peak III migrates very close to natural toxin D_1. The E. coli growth inhibition assay also shows that peak III is a biologically active toxin, whose specific activity is about 43 percent. Peak I has no activity and peak II is possibly a mixture of I and III and shows an inhibition activity of about 18 percent.

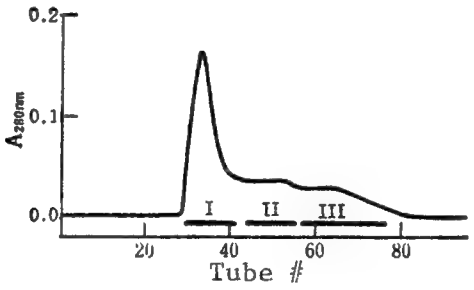

Figure 3. Chromatogram of Oxidized Synthetic Peptide on Column of Sephadex G-50

Sephadex G-50 column (2.2x83 cm); Eluant: 1N HOAc; Volume collected in each tube: 3.8 ml

The amino acid analysis data in Table 4 show that the composition of the three products is very similar and all agree with the theoretical values. This seems to suggest that they are all part of the same synthetic peptide chain but an inactive product was formed due to the different degree of cross-linking or incorrect intramolecular disulfide bridge formation during oxidation. This is further supported by the fact that activity was restored after the freeze-dried powder of peak I was reduced and reoxidized. However, it required a more stringent reducing condition possibly because the interaction between the peptide chains with a high content of hydrophobic residue creates a compact internal structure of the aggregate.

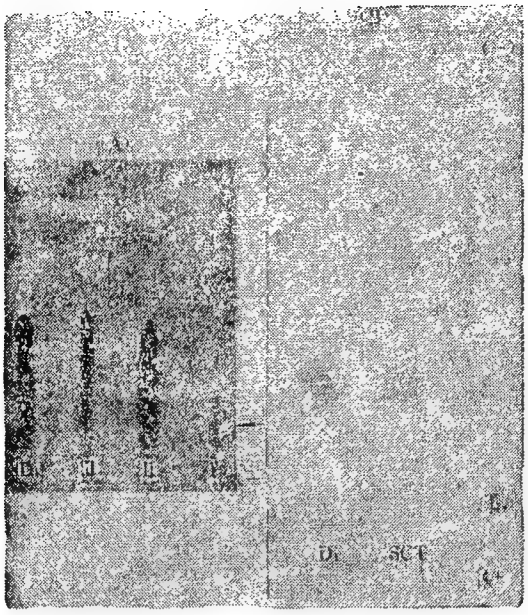

Figure 4. Diagrams of Electrophoresis on Cellulose Acetate

Electrophoretic condition: 0.04N phosphate buffer (pH 6), 250V/10cm, 5 mA, 15 minutes. (A) Electrophoretic mobilities of peaks I, II, and III from Sephadex G-50 column and natural toxin D_1; (B) comparison of the natural toxin with product (SCT) purified by CMC column

Table 4. Amino Acid Composition of the Synthetic Peptides

	RATIO	D_1	32-ϕ*	60-ϕ*	G50-I	G50-II	G50-III	CMC-V
Asp	7	6.90	5.04(5)	7.33	7.00	6.75	7.11	6.82
Thr	2	2.03	1.05(1)	1.88	1.91	1.75	2.00	2.05
Ser	3	2.89	1.71(2)	2.68	2.80	2.50	2.24	2.87
Gly	2	2.13	0.99(1)	2.08	2.37	2.29	2.22	2.20
Ala	1	1.05	0.02(0)	1.05	1.25	1.15	1.25	1.05
Cys	8**	—	—(5)	—	—	—	—	—
Val	7	6.37	4.62(5)	5.91	6.87	6.17	6.89	6.47
Met	3	2.72	0.80(1)	2.04	2.65	2.38	1.84	2.31
Ile	1	0.87	0.87(1)	0.93	0.95	0.84	0.71	1.00
Leu	6	6.04	1.83(2)	6.08	6.81	6.03	6.40	6.37
Tyr	3	2.82	1.02(1)	2.43	2.87	2.63	2.71	2.65
Phe	2	1.95	0.00(0)	1.87	2.09	1.96	2.07	2.09
Lys	9	8.89	1.88(4)	8.13	9.19	8.65	8.27	9.08
Arg	2	1.82	1.97(2)	1.88	1.86	2.05	2.07	2.09
Pro	4	4.00	2.06(2)	3.97	4.14	4.15	4.58	4.06

*Hydrolysis analysis was carried out at the designated stage of the peptide synthesis.

**Due to the presence of phenol during hydrolysis, cysteines were partially destroyed and not determined.

For further purification, the freeze-dried powder of peak III (6 mg) from the Sephadex G-50 column was dissolved in 0.05M ammonium acetate (pH 6.0) and purified by cellulose acetate ion exchange chromatography (0.8x6 cm), using a linear gradient of 0.5M NH_4OAc as eluant. The chromatogram is shown in Figure 5. The peaks in Figure 5 were collected separately, desalted through a Biogel P_2 column, and freeze-dried. The results showed that most proteins were concentrated in peak V, which corresponds to the elution position of the natural toxin D_1. Though showing a strong absorption, peak IV contains very little protein.

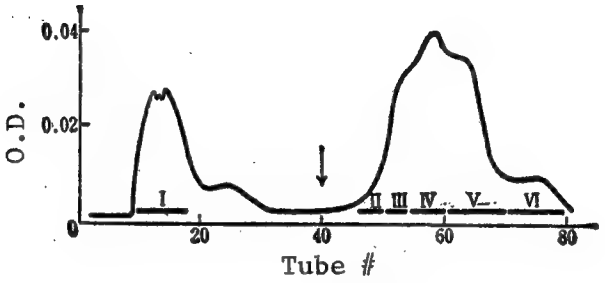

Figure 5. Purification of Synthetic Cytotoxin

Chromatography on CMC column (0.8x6 cm). Equilibration with 0.05M NH_4OAc (pH 6). Arrow indicates the beginning of gradient. Volume collected in each tube: 1.25 ml. Heavy bars indicate the portions collected.

The purified product (peak V) gives a single spot on electrophoresis at pH 6.0 with the same mobility as the natural D_1 standard (Figure 4B). Furthermore, its amino acid composition (see Table 4) also agrees with that of the toxin D_1. In the previous paper, the quantitative inhibition of E. coli on solid media by natural toxins was reported.[4] Based on that finding, the biological activity of the synthetic product was determined. The results show that it is 94 percent of the natural D_1. To study further the antigenicity of the synthetic product, we conducted a double-diffusion experiment on the synthetic toxin and the control, natural D_1, using rabbit antisera induced by a natural membrane toxin. The diffusion patterns, shown in Figure 6, are identical. This clearly indicates that the new synthetic toxin has the same antigenic characteristics as that of the natural membrane toxin. One can assume that the surface structures of these molecules are basically identical.

In summary, a new cytotoxin has been synthesized through solid-phase fragment condensation. In the previous paper,[10] the species variations of Chinese cobra cytotoxin were compared. Because the middle-section variable regions of MT-B and MT-A (SNLT and SDLT, respectively) do not contain lysine, their bacterial-inhibition activities are nearly one order of magnitude lower than those of MT-C (ATPK) or MT-D_1 (SNKM).[4] The level of bacterial inhibition by the synthetic toxin is far higher than that of MT-B and is about the same as MT-D_1. This confirms the importance of the basic residue in the middle variable region with respect to the function.

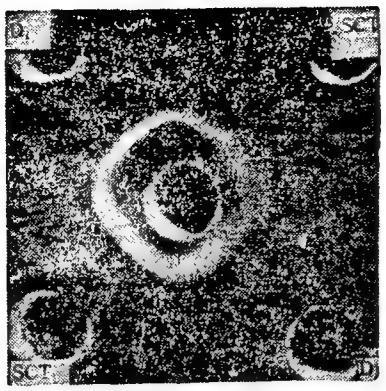

Figure 6. Immuno-diffusion Pattern of Synthetic Membrane Toxin

Center well: antisera; other samples are described in the legend of Figure 4.

In the literature report on the synthesis of a Taiwanese cobra cardiotoxin, a 60mer, by the solid-phase step-wise method, the yield was 31 percent and the purification required CMC column separation followed by affinity chromatography.[18] Our yield, as described here, is 59 percent. This shows the superiority of the approach of solid-phase fragment condensation to the step-wise method. Judged from the total synthesis procedure, the main reasons for the lowered overall yield are the high content of sulfur-containing residues and the rearrangement of disulfide bridges. It seems that the oxidation condition needs improvement. Treating with HF at 0°C for 1 hour only removes 70-80 percent of the S-benzyl protecting group[19] so it is necessary to proceed at 20°C for a short period of time. Even so it is inevitable that a substantial amount of incompletely deprotected benzyl derivatives will hide under the overoxidized product peak I, with the consequence of diminished solubility of the peptide chain and a lowered yield. The DMB group has the advantage of being easily removed by HF, but the synthetic product cannot be completely cleaved from the resin. Whether it is due to the side reaction caused by the sulfonation of the sulfur atoms awaits further investigation.

Experimental

All the solvents were treated with a 5A molecular sieve to remove moisture. Unless otherwise specified, the 37 system made up of the following freshly prepared ingredients was used for TLC: 30 percent of pyridine:acetic acid: water = 4:1:1.5 and 70 percent of butyl acetate:isopropanol = 10:4. Cytotoxin D_1 was purified in our lab from Guangdong cobra venom.[9] The salicylaldehyde method used to determine resin-bound amino group is described in the literature.[16] The cellulose acetate membrane is the product of Hunan Chemical Reagents Supply of Shanghai.

BOC-Asn-ϕ. This is prepared by literature methods.[11,15] One gram of 1 percent cross-linked polystyrene resin (Pierce Co, chlorine content: 1.03 meq/g) was converted into its thioether derivative. Then, a solution

of BOC-Asn-ϕ (230 mg) in dioxane was added. The exchange took place at 30°C for 40 minutes. After filtration, evaporation, and vacuum drying at 80°C for 9 hours, the product was deprotected with 50 percent TFA/CH_2Cl_2. The free amino group content was determined as 0.702 mmol/g, a 70 percent yield of esterification.

Solid-phase Peptide Coupling. See previous paper[11] for general procedure. Modifications were made as follows: 1) Every time the N-terminal protecting group was being removed, 1,2-ethanedithiol (0.15 ml) was added in addition to methionine in order to keep the sulfur-containing residues from being destroyed. 2) At the same time exposed amino groups were assayed, and the resin-bound peptide was vacuum-dried for 3 hours and weighed to determine the weight gain after each condensation step.

Asn-ϕ. It was condensed first with BPOC-Cys(BZL)-OH (3.5 equivalents) and then with BOC-Arg(TOS)-OH (3 equivalents). After capping off unreacted amino groups with acetic anhydride and removing BOC group with acid, a tripeptide resin HCl-Arg(TOS)-Cys(BZL)-Asn-ϕ containing 0.547 mmol/g of the free amino group was obtained. The tripeptide resin (291 mg) was used as starting material for the subsequent couplings with the 13 peptide fragments. The conditions and results are listed in Table 3. The final yield: 1.01 g of resin-bound 60mer.

HF Treatment. Equipment was as described in the previous paper[11] with slight modifications, namely a gas outlet was added on the cover of reactor B and a PTFE valve attached to the outlet to control the reaction. A mixture of peptide resin (190 mg), methionine (20 mg), and redistilled p-cresol (0.5 ml) was stirred at 4-6°C. At the end of the reaction, HF was removed by passing dry nitrogen through for 15 minutes. The ether precipitate was centrifuged and washed twice with anhydrous ether. To the centrifuge tube was then added 2 ml of 0.02M Tris buffer containing 6M guanidine chloride (degassed by bubbling nitrogen through for 10 minutes) and 0.2 ml of mercaptoethanol. The pH was adjusted to 7.5 with methylamine and the tube was evacuated and then filled with nitrogen. After repeating four more times it was tightly capped to assure an anaerobic condition. The product was reduced at 37°C for 40 minutes and the resin was removed by centrifugation. The supernatant was immediately applied to a column of Biogel P_6, which was pre-equilibrated for 48 hours with oxygen-free 0.1N HOAc, to remove high polymers and salts. The major peak was collected under nitrogen and freeze-dried to get 62 mg of reduced 60mer peptide.

Oxidation and Separation. The freeze-dried powder of the above 60mer was quickly dissolved in 200 ml of 0.2M phosphate buffer (pH 6.8) and oxygen-free distilled water was added so that the final solution (1,150 ml) contained 0.026M phosphate buffer, 54 ug/ml of polypeptide, and 0.174 percent of of NaN_3, pH 7.08. Let stand at 25°C for oxidation.

Chromatographic Separation. The oxidation solution, after being centrifuged to remove traces of precipitate, was concentrated under reduced pressure and dialyzed against 1N HOAc to remove the bulk of salts. The solution was separated on a column of Sephadex G-50 (2.2x85 cm) and eluted with 1N HOAc.

The result is shown in Figure 3. Peak III was pooled and freeze-dried into powder (5 mg). The dry powder (4 mg) was dissolved in water (1 ml) and passed through a CMC exchange column (1x6 cm), which was equilibrated with 0.05M NH_4OAc and eluted with a linear gradient of 0.05M and 0.5M NH_4OAc (pH 6, 30 ml each). The eluants were collected and desalted with Biogel P_2 to get the active product from peak V (1 mg).

Activity Assay. The procedure of Qu, et al.[20] was followed. Samples (3 µl) were applied on an E. coli covered plate, which was incubated at 37°C for 24 hours. The inhibition zones were them measured. Based on the diameters of the inhibition zones from three or four dilutions of the same sample, a straight line was obtained by plotting the diameters versus logarithmic toxin concentrations. When compared with the natural product, the activity of the samples could be calculated.

Immunological Assay. The cytotoxins isolated in our lab were mixed with Freund complete adjuvant and injected subcutaneously in the neck of rabbit at an interval of 2 weeks. The initial dosage was 1.5 mg/kg, which was gradually raised to 2.2, 3.0, and 3.5 mg/kg. Then it was maintained at 2 mg/kg. Ten days after the seventh injection, blood was drawn and antisera isolated. The immuno-diffusion assays were conducted with the center well containing 15 µl of 4-fold diluted antisera and samples and natural products placed around it as shown in Figure 6.

Z-Leu-Lys(Z)-OH·CHA(I-1). HCl·Lys(Z)-OCH_3 (5 grams, 15.1 mmol) in THF (20 ml) was adjusted to pH 8 at -5°C with triethylamine (2.1 ml) and was quickly mixed with a THF solution (10 ml) of Z-Leu-OH (3.76 mg, 15.1 mmol). After thorough mixing, DCCI (3.38 grams, 16.6 mmol) was added. The reaction was brought up to room temperature after 1.5 hours at -10°C. The substituted urea was filtered off and the filtrate evaporated to dryness. The residue was dissolved in EA and washed with regular acid and alkaline solution, dried and concentrated. The concentrate was crystallized from PE. Yield: 3.6 grams (55 percent); mp 88-90°C; homogeneous by TLC; R_f 0.96. This dipeptide ester was saponified in 30 ml of methanol, whose pH was maintained at 9 with NaOH. After saponification, the product was extracted with ether, acidified with dilute HCl, and washed with water. It was converted into CHA salt and crystallized. Yield: 4.6 grams of I-1 (88 percent); R_f 0.66; mp 110-112°C; homogeneous by TLC.

BOC-Asn-Lys(Z)-OCH_3(I-2). HCl·Lys(Z)-OCH_3 (4.6 grams, 14 mmol) in THF (20 ml) was adjusted to pH 8 with triethylamine (1.96 ml), mixed with a THF solution (30 ml) of BOC-Asn-ONP (4.1 grams, 12 mmol), and stirred at 37°C for 48 hours. After being concentrated, the product was precipitated with an ample amount of water and filtered. The solid was washed with 1M citrate, water, and ethyl acetate successively and recrystallized from methanol to give 3.1 grams of I-2. Yield: 51 percent; R_f 0.84; mp 181-182°C.

BOC-Cys(DMB)-Asn-Lys(Z)-OH(I-3). Peptide I-2 (3.1 grams, 6.09 mmol) was dissolved in TFA (15 ml) and CH_2Cl_2 (15 ml) and allowed to stand at 30°C for 30 minutes. The reaction was evaporated and the oil was solidified with diethyl ether. The solid was redissolved in 15 ml of THF and neutralized with 0.85 ml

of triethylamine. Also BOC-Cys(DMB)-OH (6.95 mmol) was dissolved in 10 ml of THF and converted into its mixed anhydride by reacting with triethylamine (0.85 ml) and IBCF (0.92 ml) at -10°C. After 20 minutes, the above dipeptide ester was added and the reaction continued at 10°C for 2 hours. The product was washed in the same manner as described for I-2 and crystallized from 50 ml of hot methanol to give 3.6 grams of white crystals. Yield: 82 percent; homogeneous by TLC; R_f 0.74; mp 158-160°C. The protected tripeptide ester (1.45 grams, 2 mmol) was saponified with NaOH in a mixture of dioxane (25 ml) and water (3 ml) until the ester spot disappeared on TLC. The solution was concentrated and acidified in an ice bath. The precipitate was washed with 0.01N HCl and EA, followed by water until neutral. Recrystallization in methanol gave 0.95 gram of I-3. Yield: 66 percent; R_f 0.44; mp 205-207°C; carboxyl assay: 100 percent.

Z-Leu-Lys(Z)-Cys(DMB)-Asn-Lys(Z)-OH(I), MW1143.39. After being acidified (HCl) in EA, desalted, washed, and dried, the dipeptide I-1 (1.63 grams, 2.6 mmol) was dissolved in 5 ml of THF and reacted with 300 mg of HOSu and 536 mg of DCCI at -10°C in an ice bath. After overnight in a refrigerator, the substituted urea was filtered off and filtrate-evaporated to dryness. And the tripeptide I-3 (1.2 grams, 1.7 mmol) was treated with 16 ml of 50 percent TFA/CH_2Cl_2 at 30°C for 30 minutes. The reaction was concentrated and the TFA removed by diethyl ether extraction. The concentrate was neutralized with triethylamine at -5°C in a 1:1 mixture of DMF and DMSO. Finally this solution was mixed with the activated ester prepared above and stirred for 2 days at 30°C, when the thick mixture became fluid. The syrup, obtained by evaporation under reduced pressure, was triturated with 0.02N HCl (100 ml), water, and EA. Recrystallization with methanol gave 690 mg of product I. Yield: 36 percent; homogeneous by TLC; R_f 0.7; mp 214°C. The elemental and amino acid composition analysis data are listed in Table 2.

Z-Pro-Leu-OH(II-1). A solution of THF (20 ml) containing Z-Pro-OH (2.5 grams, 10 mmol) was mixed with a solution of HCl·Leu-OCH_3 (1.8 grams) and triethylamine (1.4 ml, 10 mmol) in THF (10 ml) in an ice bath and DCCI (2.06 grams, 10 mmol) was added. After overnight reaction, the solution was filtered and the filtrate evaporated to dryness. The residue was redissolved in EA and washed with dilute acid and base. Evaporation and crystallization from PE gave 3.2 grams of crystals (8.5 mmol). Yield: 85 percent; homogeneous by TLC; R_f 0.90; mp 66°C. Z-Pro-Leu-OCH_3 (6 grams, 16 mmol) was saponified with NaOH in 40 ml of methanol and the reaction extracted with water, acidified to pH 3.0 with HCl, and allowed to stand in a refrigerator. The precipitate was filtered and washed thoroughly with water to give 4.73 grams (13.1 mmol) of Z-Pro-Leu-OH(II-1). Yield: 82 percent; homogeneous by TLC; R_f 0.66. Elemental analysis: C, 63.06 (62.96); H, 7.42 (7.23) (numbers in parentheses denote calculated values).

Z-Pro-Leu-Phe-OCH_3(II-2). A solution of HCl·Phe-OCH_3 (2.15 grams, 10 mmol) in THF (20 ml) was neutralized with triethylamine (1.4 ml) in an ice bath and then mixed with a solution of peptide II-1 (3.62 grams, 10 mmol) in THF (5 ml). Condensation was carried out by adding immediately 2.06 grams of DCCI. At the end of the reaction, the solution was filtered and the filtrate evaporated to dryness. The residue was dissolved in 100 ml of EA and washed

successively with diluted acid and base. After drying and evaporation, the residue was solidified by adding PE to get 4.31 grams of II-2. Yield: 82 percent; homogeneous by TLC; R_f 0.865. Elemental analysis: C, 66.18 (66.51); H, 7.05 (7.12); N, 7.81 (8.12).

HCl·Val-Pro-Leu-Phe-OCH_3(II-3). Hydrogenation of II-2 (4.3 grams) was done in methanol in the presence of HCl/HOAc with Pd/C as a catalyst. The deprotected tripeptide ester was converted into its HCl salt in EA to get 2.9 grams of crystals (6.8 mmol), which was added to a solution of THF (30 ml) and triethylamine (1.4 ml) at 0°C, followed by the additions of Z-Val-OH (1.74 grams, 6.9 mmol) and DCCI (1.42 grams). At the end of the reaction, the solution was filtered and the filtrate evaporated to dryness. The residue was dissolved in EA, washed as before, and crystallized from E-PE. All of the product was subjected to catalytic hydrogenation in methanol. The solution was evaporated and the resulting oil crystallized from CH_3OH-E to get 2.5 grams of II-3. Yield: 58 percent; homogeneous by TLC; amino acid composition: Pro 1.03, Val 0.97, Leu 0.95, Phe 1.05.

BPOC-Leu-Val-Pro-Leu-Phe-OH(II), MW840.09. 2.3 grams of II-3 (4.35 mmol) in 10 ml of THF were neutralized with 0.63 ml of triethylamine and mixed with a THF solution (10 ml) containing BPOC-Leu-OH (1.67 grams, 4.5 mmol) at 0°C. DCCI (0.94 grams) and traces of BTA were added immediately and the reaction was continued overnight. The substituted urea was filtered off and the filtrate evaporated and redissolved in EA. Crystallization in ether after a rountine work-up yielded 2.86 grams (3.4 mmol) of protected peptide ester. Yield: 78 percent; homogeneous by TLC; R_f 0.78; mp 107-109°C. The elemental analysis and amino acid analysis are satisfactory (see Table 2). The pentapeptide ester (1 gram, 1.19 mmol) was saponified in methanol, using NaOH to maintain pH at 10 until the pH no longer changed. With vigorous stirring in an ice bath, it was acidified to pH 4 with 0.1N HCl and was then diluted with an ample amount of water. The precipitate was collected and the filter cake washed with water and vacuum-dried to get 950 mg of peptide II. Homogeneous by TLC; R_f 0.64; mp 120-122°C. The peptide was used directly for solid-phase condensation.

TFA·Lys(Z)-Thr(BZL)-OBZL(III-1). With vigorous stirring in an ice-salt bath, a THF solution (15 ml) containing BOC-Lys(Z)-OH (4.17 grams, 11.5 mmol) was reacted with 1.27 ml of NMM and 1.51 ml of IBCF for 20 minutes. A 5-ml solution of THF containing 4 grams of Thr(BZL)-OBZL was added and stirring was continued overnight. The next day, THF was removed under reduced pressure and the residue dissolved in 50 ml of EA. The solution was washed successively with 2 percent citrate, water, 5 percent $KHCO_3$, and water, three times each, and dried over Na_2SO_4. Evaporation under reduced pressure gave 7.41 grams of oily material. Yield: 97 percent; homogeneous by TLC; R_f 0.9. This oily material was treated with 50 percent TFA/CH_2Cl_2 at 20°C for 40 minutes. After evaporation under reduced pressure, the residue was triturated with diethyl ether and recrystallized from CH_3OH-E. mp 136°C.

BPOC-Tyr(DCB)-Lys(Z)-Thr(BZL)-OH·CHA(III), MW1131.23. BPOC-Tyr(DCB)-OH (1.41 grams, 2.44 mmol) was dissolved in THF (10 ml) and converted into its mixed anhydride with 0.27 ml of NMM and IBCF in the same manner as described for

III-1 before reacting with the triethylamine neutralized dipeptide III-1 (2.44 mmol). After being washed and twice recrystallized in EA-E, 1.76 grams of the tripeptide ester were obtained. Homogeneous by TLC; R_f 0.97; mp 96°C. The tripeptide ester was dissolved in 80 percent acetone and saponified with 1N NaOH. The pH was maintained at 8-9. After the reaction, acetone was removed under reduced pressure and the residue acidified with citric acid in an ice bath. The product was extracted into EA, which was washed with water and dried over Na_2SO_4. The CHA salt of III was prepared in diethyl ether to get 1.63 grams (1.44 mmol) of crystals. Yield: 91 percent; homogeneous by TLC; R_f 0.78; mp 140°C. See Table 2 for amino acid analysis.

Z-Pro-Ala-Gly-OCH_3(IV-1). Z-Pro-Ala-OH was prepared by the literature method,[21] mp 158-160°C. Its mixed anhydride was prepared as before from a solution of Z-Pro-Ala-OH (11 grams, 34.4 mmol) in THF (50 ml) and DMF (3 ml), triethylamine (5.3 ml), and IBCF (4.6 ml). An aqueous solution (25 ml) containing 6.2 grams of HCl·Gly-OC_2H_5 (41.3 mmol) and 6.3 ml of triethylamine was then added. After the reaction, the product was extracted with chloroform and washed. Crystallization from ethanol-PE gave 6.23 grams of IV-1. Yield: 45 percent; R_f 0.8; mp 138-139°C. Elemental analysis: C, 59.67 (59.24); H, 6.87 (6.71); N, 10.42 (10.36).

BPOC-Cys(BZL)-Pro-Ala-Gly-OH(IV), MW674.83. Hydrogenation of 2.85 grams of IV-1 (7 mmol) in methanol was done for 3 hours in the presence of Pd catalyst, when TLC showed the completion of deprotection. The solution was filtered and evaporated. The residue was dissolved in 20 ml of THF/DMF mixture and 0.8 ml of NMM was added to free the amino group. This solution was immediately mixed with 20 ml of a THF solution containing 7.1 mmol of BPOC-Cys(BZL)-OH. With stirring in an ice bath, DCCI (1.5 grams, 7.3 mmol) and BTA (0.24 gram) were added. The stirring was continued overnight at low temperature. After filtration and evaporation under reduced pressure, a syrup resulted, which was partitioned between EA and 1N citrate solution. After routine work-up, the organic phase was solidified in PE to get 4.31 grams of the product tetrapeptide ester. Yield: 91.3 percent; R_f 0.77. 2.1 grams of the product (3.3 mmol) were saponified with NaOH in 20 ml of dioxane for 20 hours. Purification by a column of silica gel with 10 percent ethanol/EA as eluant gave 1.46 grams of the chromatographically pure protected tetrapeptide. Yield: 65.5 percent; R_f 0.54; mp 56-68°C. See Table 2 for elemental analysis and amino acid analysis.

Z-Asn-Leu-OCH_3(V-1). In an ice bath, 1.8 grams (10 mmol) of HCl·Leu-OCH_3 were extracted into EA to form free ester, which was dissolved in a mixture of THF/DMF (2:1, 30 ml) and reacted with 3.9 grams of Z-Asn-ONP (10 mmol) at 25°C for 5 days. Solvent was removed under reduced pressure and the residue dissolved in large quantity of EA and washed with 1N NH_4OH until colorless and then washed thoroughly with water and 0.5N HCl to get 1.96 grams of dry powder. Yield: 50 percent; mp 176-178°C; homogeneous by TLC; R_f 0.81; MW 393.45. Elemental analysis: C, 58.27 (58.00); H, 6.87 (6.92); N, 10.86 (10.68).

BPOC-Lys(ZCL)-Asn-Leu-OH(V), MW780.34. BPOC-Lys(ZCL)-OH (3.27 grams, 5.9 mmol) was dissolved in 30 ml of THF and neutralized with 0.65 ml of NMM. It was converted into a mixed anhydride by reacting with 0.77 ml of IBCF at -30°C with vigorous stirring. After 20 minutes, dipeptide ester H-Asn-Leu-OCH_3 (5.9 mmol) in 30 ml of THF was added and stirring was continued overnight. Solvent was evaporated under reduced pressure and the residue dissolved in EA and followed by the routine acid and base washing. After drying, 4.5 grams of the tripeptide ester were obtained. Yield: 95.9 percent; mp 130-132°C; homogeneous by TLC; R_f 0.92. The tripeptide ester (1 gram) was dissolved in 80 percent aqueous dioxane (20 ml) and the pH was maintained at 9-10 at 30°C with 0.87N KOH. After saponification for 19 hours, dioxane was evaporated under reduced pressure and the remaining liquid acidified to pH 4 with HCl in an ice bath. Extraction with EA and crystallization from EA-E gave 870 mg of the tripeptide V. Yield: 88 percent; homogeneous by TLC. See Table 2 for analysis data.

BOC-Tyr(DCB)-Lys(ZCL)-OH·CHA(VI-1). A solution of BOC-Tyr(DCB)-OH (3 mmol) and NMM (0.31 ml) in THF (15 ml) was cooled to below -10°C and IBCF (0.37 ml) was added with vigorous stirring to form a mixed anhydride. A mixture of H-Lys(ZCL)-OH (882 mg, 2.8 mmol) in 15 ml THF, 7 ml H_2O, and 0.31 ml NMM was then added. The reaction was stirred at a low temperature overnight. Solvent was evaporated under reduced pressure and the residue was partitioned between EA (100 ml) and water (40 ml), which was acidified with dilute HCl in an ice bath. The ester layer was washed with water and dried over Na_2SO_4. The volume was reduced to 6 ml and 0.5 ml of CHA added. The precipitate was recrystallized from CH_3OH-E to get 1.1 grams of CHA salt of the dipeptide VI-1. Yield: 47 percent; mp 118-120°C; homogeneous by TLC; R_f 0.72, MW 836.28. Elemental analysis: C, 58.98 (58.89); H, 6.48 (6.38); N, 6.86 (6.70).

BPOC-Cys(BZL)-Tyr(DCB)-Lys(ZCL)-OH·CHA(VI), MW1167.72. BPOC-Cys(BZL)-OH (1 mmol) was dissolved in 8 ml of THF, mixed with 0.11 ml of NMM and cooled to -10°C. With vigorous stirring, 0.13 ml of IBCF was added and the reaction proceeded at -10°C for 20 minutes. Then free dipeptide solution was added to the reaction vessel. The preparation of free dipeptide was as follows: The dipeptide VI-1 was treated with 5 ml of 2N HCl/HOAc at 20°C for 1.5 hours to remove the BOC protecting group. Solvent was evaporated under reduced pressure and the residue was triturated with diethyl ether, which was then evaporated to remove residual HCl, redissolved in 16 ml of 50 percent aqueous THF, and neutralized with 0.11 ml of NMM. After removal of solvent under reduced pressure, the product was partitioned between water and EA, which was carefully acidified in an ice bath with 1N HCl. The aqueous phase was discarded and the organic phase was washed with brine until neutral and dried over Na_2SO_4. After evaporation, the glassy solid was converted into CHA salts in EA and recrystallized from methanol-E to get 1.04 grams of product VI. Yield: 88 percent; homogeneous by TLC; R_f 0.77. MW of the CHA salt of protected tripeptide containing one molecule of methanol: 1,199.76. See Table 2 for analysis data.

BOC-Val-Ser(BZL)-OCH_3(VII-1), MW408.50. BOC-Val-OH (4.4 grams, 20 mmol) was dissolved in THF (40 ml) and cooled to -15°C. With vigorous stirring, 2.25 ml

of NMM and 3 ml IBCF were added in order to prepare the mixed anhydride. After 20 minutes, the DMF solution of H-Ser(BZL)-OCH_3 (26 mmol) was added. The reaction was warmed up to room temperature and continued for 2.5 hours. Solvent was evaporated under reduced pressure and the product dissolved in EA and washed 3-4 times with water, 1M citrate, brine, 8 percent $NaHCO_3$, and brine again. The organic phase was evaporated to dryness and crystallized from PE to get 5.0 grams of long needle-shaped crystals of VII-1. Yield: 61 percent; homogeneous by TLC; R_f 0.91; mp 70-72°C. Elemental analysis: C, 61.55 (61.74); H, 7.52 (7.88); N, 7.05 (6.86).

BOC-Met-Val-Ser(BZL)-OCH_3(VII-2), MW677.87. Following the above procedure (VII-1), BPOC-Met-OH (10 mmol) in 25 ml of THF was converted into its mixed anhydride derivative with 1.12 ml of NMM and 1.4 ml of IBCF, which was then reacted with the dipeptide VII-1, whose N-terminal protecting group had been removed by TFA and was neutralized with NMM. After washing, the product was purified by repeated crystallization in EA-PE to get 5 grams of the tripeptide VII-2. Yield: 74 percent; mp 100-102°C; homogeneous by TLC; R_f 0.96. Elemental analysis: C, 66.06 (65.55); H, 6.97 (6.98); N, 6.39 (6.19).

BPOC-Met-Phe-OH·CHA(VII-3), MW633.86. HCl·Phe-OCH_3 (3.2 grams, 15 mmol) was dissolved in a mixture of THF (15 ml) and DMF (15 ml) and neutralized with 1.7 ml of NMM. The solution was added to a THF solution (30 ml) of BPOC-Met-OH mixed anhydride (prepared from 5.9 grams of BPOC-Met-OH, 1.7 ml of NMM, and 2.1 ml of IBCF). Following the procedure for VII-1, 7.5 grams (13.7 mmol) of oil were obtained. Yield: 91 percent; homogeneous by TLC; R_f 0.95. The dipeptide ester was dissolved in 75 ml of dioxane and pH maintained at 9-10 with 0.97N KOH until TLC showed complete saponification. Solvent was removed under reduced pressure and the residue redissolved in EA, washed with brine, and dried. After concentration, it was converted into CHA salt in diethyl ether to get 5 grams of the product (52.6 percent). Recrystallization from CH_3OH-E gave 4.1 grams of the dipeptide salt. Homogeneous by TLC; R_f 0.8; mp 118-120°C. Elemental analysis: C, 67.92 (68.21); H, 7.45 (7.47); N, 6.50 (6.62).

BPOC-Met-Phe-Met-Val-Ser(BZL)-OH·CHA(VII), MW1041.40. The tripeptide VII-2 (2.0 grams) was dissolved in 13 ml of CH_2Cl_2 and deprotected with 0.2 ml mercaptoethanol and 3 ml TFA at 30°C for 1 hour. Diethyl ether was then added and the mixture centrifuged to collect the precipitate, which was washed with diethyl ether and dried to give 1.53 grams (2.85 mmol) of the TFA salt of the tripeptide ester (R_f 0.78). TLC showed that the deprotection was complete. Before coupling, it was neutralized and desalted to form free ester, which was then dissolved, together with 1.75 grams (3.28 mmol) of BPOC-Met-Phe-OH(VII-3), in 10 ml of THF. DCCI (710 mg in 3 ml THF) was added dropwise at 0°C with stirring. After 10 minutes, the reaction was run at 14°C overnight. DCU was filtered off and the filtrate evaporated to dryness under reduced pressure. The residue was dissolved in EA and followed by routine acid, base washing. The product was soluble in diethyl ether but failed to crystallize. It could be purified by a column of silica gel (1.6x40 cm). The eluant was 50 percent EA-E, and 700 mg of TLC pure pentapeptide ester were obtained (R_f 0.96). The pentapeptide (500 mg) was dissolved in 5 ml of methanol and saponified with 0.95N KOH overnight. The

reaction was acidified to pH 4 directly with 1N HCl in an ice bath. The precipitate was collected by filtration and the filter cake washed with brine. After vacuum drying, it (free acid of VII, homogeneous by TLC, R_f 0.83) was used directly. For storage, it was converted into its CHA salt in EA. The free acid form could also be crystallized by using CH_3OH, mp 168-170°C. See Table 2 for amino acid analysis and elemental analysis data.

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MICROQUANTITATIVE ANALYSIS OF DONSEY PEPTIDES

Beijing SHENGWUHUAXUE YU SHENGUWUWULI JINZHAN [BIOCHEMISTRY AND BIOPHYSICS] in Chinese No 3, Jun 86 pp 69-72

[Article by Pan Xiaoping [3382 1420 1627], Cai Jinlian [5591 6855 5571], Zhang Binghai [1728 3521 3189], Wang Deling [3769 1795 1545], Chemistry Teaching and Research Laboratory, Shanghai Medical University; paper received 17 December 1985]

[Text] Dansey chloride reacts with peptide forming DNS-peptide. It is highly fluorescent and stable to acid hydrolysis. Dansyl chloride is now widely employed for the determination of n-terminal amino acid of peptides and proteins and for the microquantitative analysis of amino acids in the biological samples. In recent years, the dansyl chloride procedure has been used for measuring the content of small peptides in the spinal fluids and brain tissues. This technique is highly sensitive, the fluorescene intensity is stable, there is also a good graphic correlation, and the results are repeatable too. We now introduce our experimental results as follows.

Experiments and Results

1. Dansylation of peptides

Leu-enkephalin (LEK) and Naga used in the experiment were synthesized by the Biochemistry Institute of the Chinese Academy of Sciences. DNS-Cl is a product of BDH. The preparation of DNS-peptide is according to the procedure of Gray (1). Twenty μg of peptides were transfered to a small glass test tube. A 30 μl of 0.2 N $NaHCO_3$ solution (pH was adjusted to 9.5. The solution was made by using ammonia free distilled H_2)) was added to the tube. To this mixture was then added an equal volume of the dansyl chloride acetone solution (2.5 mg/ml). The tube was covered tightly and the reaction was allowed to proceed for an hour in a 37°C water bath, or stored in the dark overnight. The remaining dansyl chloride was hydrolyzed by adjusting the pH to around 4 with 1 N HCl. The solution was allowed to dry in vacuum.

2. The purification of DNS-peptide

The vacuum dried DNS-peptide was dissolved in a small amount of pure methanol and transferred to a silica Gel G thin plate (GF 254, Type 60, Merk. 10x10 cm) for chromatograph to separate the reaction by by-products, DNS-OH and DNS-NH_2.

Two solvent systems were employed for the development of DNS-peptide. The first one was n-butanol:glacial acetic acid: H_2O = 4:1:1, the second system was n-heptane: n-butanol:glacial acetic acid = 3:3:1. After drying, the peptides were located on the thin plate with a 365 nm uv light. The fluorescence bands were transferred together with the silica get G into a small chromatotube. The peptides were eluted with pure methanol and dried in vacuum. They were then extracted once more with pure chloroform, dried in vacuum again. The final product is pure DNS-peptide.

3. Fluorescence and excitation measurements of DNS-peptides

Purified Leu-enkephalin, Naga, and glycel glycine were separately disolved in methanol to form a 10^{-12} M solution. Fluorescence and excitation measurements were carried out in a MPF-4 spectroflurometer. Results are shown in figures 1, 2, and 3.

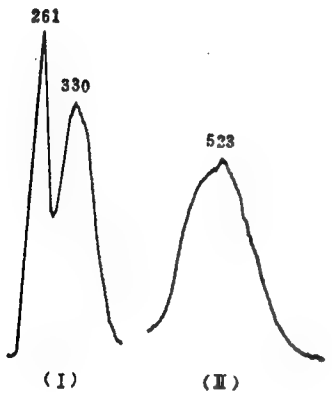


Figure 1.

Excitation (1) and flourescence (11) spectra of DNS-Leu-enkephalin

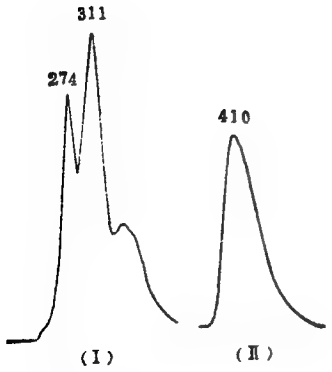
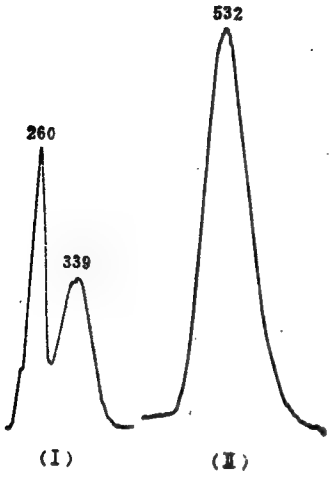


Figure 2.

Excitation (1) and fluorescence (11) spectra of DNS-Naga



Excitation (1) and
Fluorescence (11) spectra
of Glycyl glycine

Figure 3.

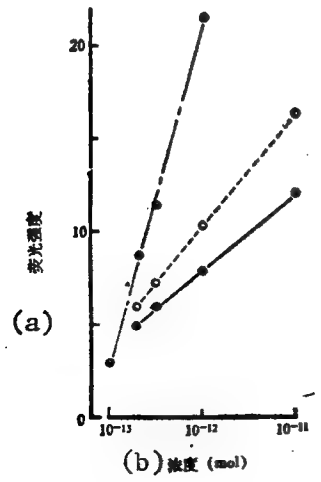
4. Fluorescence emission spectra of DNS-peptides

Methanol solutions of DNS-Leu-enkephalin, DNS-Naga, and DNS-glycyl glycine in concentrations of 0.25×10^{-12}M, 0.5×10^{-12}M, 1×10^{-12}M, 0.5×10^{-11}M and 1×10^{-11}M were made for quantitative measurement of fluorescence in a 3 ml photometric cell. The excitation and fluorescence wavelengths used for the measurements are indicated in table 1. The standard curves for the quantitative measurements of the fluorescence intensity are shown in figure 4.

Table 1. Maximum excitation and fluorescence wavelengths of DNS-peptides

	DNS-LEK*	DNS-Naga	DNS-Glycyl glycine
Maximum excitation-wavelength (nm)	339	311	330
Maximum fluorescence-wavelength (nm)	523	410	532

*Leu-enkenphalin



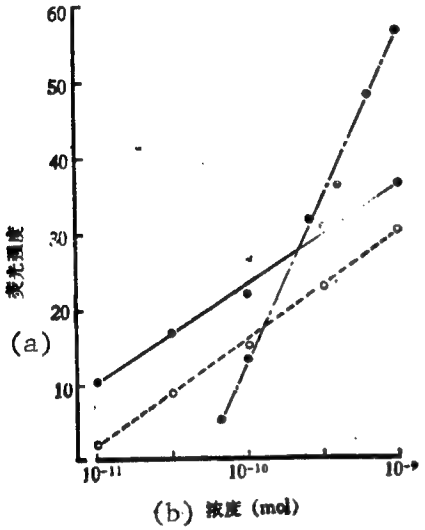
Standard curves for the proportionality between the fluorescence intensities and the concentrations of DNS-peptides

- DNS-Naga
- DNS-Glycyl glycine
- DNS-Leu-enkephalin

(a) Fluorescence intensity
(b) Concentration (mol)

Figure 4.

Methanol solutions of DNS-Leu-enkephalin, DNS-Naga, and DNS-glycyl glycine at concentrations of $1x10^{-11}M$, $0.5x10^{-10}M$, $1-x10^{-10}M$, $0.5x10^{-9}M$ and $1x10^{-9}M$ were applied to the polyamide sheet with a 1 µl quantitative micropeptide and proceeded with two dimensional chromatograph. Two solvent systems were employed: formic acid: H_2) = 1.5:100 (v/v), and n-butanol:pyridine:acetic acid - 5:5:1 (v/v/v). The DNS-peptide spots were located by using a µv lamp at a wavelength of 254 nm.



Standard curves for the proportionality between the fluorescence intensities and the concentrations of DNS-peptides on polyamide sheet

•——• DNS-Naga
o——o DNS-Glycyl glycine
o- - -o DNS-Leu-enkephaline
(a) Fluorescence intensity
(b) Concentration (mol)

Figure 5.

6. Measurement of the content of Naga in the rabbit spinal fluid

According to the method of our laboratory for collecting the spinal fluid from rabbit, a stainless steel tube with a 0.9 mm diameter was inserted in the metencephalon of the rabbit. Spinal fluid was collected 5 days later and stored immediately in the freezer (-20°C). Dansylation of Naga was carried out by drying 20 µl of spinal fluid in vacuum. After drying, the sample was dissolved in 20 µl of 012 N $NaHCO_3$ and 20 µl of DNS-Cl in acetone. Since the protein concentration in the spinal fulid is low, no additional treatment is needed before dansylation. After drying, the dansylated sample was dissolved in 10 µl of pure quantitative micropipette for two dimensional chromatograph as described in step 5. The fluorescence spot of DNS-Naga was visualized by uv light at a wavelength of 254 nm. Quantitative determination of fluorescence was carried out the next day. The excitation and fluorescence wavelengths were 254 nm and 311 nm respectively. The content of Naga was determined by measuring the area of the graph. The results indicate that the average Naga content of rabbit spinal fulid is 24.2 n mole/100µl. The fluorescence spot of Naga together with the sheet was put into a small chromatograph tube and rinsed with methanol. The sample was diluted to 3 ml with methanol for the measurement of Naga content. Result indicates that the content of Naga is 25.8 n mole/100µl. The results basically agree with each other.

7. The recovery experiment

Rabbit spinal fluid was collected into 10 tubes with 20 ul/tube. Two tubes were used as control, and each of the other 8 tubes were divided into 4 duplicate sets. Into each set of tubes were added 0.2, 0.4, 0.8 and 1.6 n mole of Naga respectively. After drying in vacuum before and after dansylation, the samples were dissolved in 10 µl of pure methanol and transferred to a polyamide sheet for two dimensional chromatograph as described above. The fluorescence spots were located and the content of Naga were determined by fluorescence measurement in a spectrofluormeter. The amount of Naga recovered can be obtained by subtracting the content of Naga in the control tubes from that of the sample tubes. Table 2 shows that the recovery from the thin layer chromatograph sheet averages 94.33 percent, agreeable with the experimental requirement.

Table 2. Percent recovery of Naga

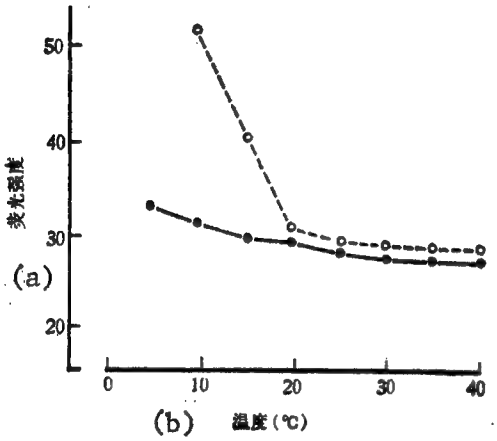
		Sample tube			
	Reference tube	1	2	3	4
Quantity of Spinal-fluid (µl)	20	20	20	20	20
Quantity of Naga-added (in mole)	-	0.2	0.4	0.8	1.6
Percent recovery	-	94.85	97.70	94.00	90.75
Average percent-recovery (%)	94.33				

Discussion

The most commonly used fluorescence reagents for the fluorometric analysis of peptides are fluorescent amide, fluorodinitrobenzene, and dansyl chloride etc. After reacting with small peptides, dansyl chloride produces the highest fluorescence intensity. The fluorescence intensity of DNS-peptides is 100 times stronger than peptides labeled with fluorescent amide. The experimental conditions for dansylation of small peptides are mild. Some of the peptides still retain their biological activities after dansylation. Since DNS-peptides are quite stable to acid hydrolysis, after quantitative measurement the DNS-peptide solution or spot can be recovered and further used for amino terminal anslysis, amino acid composition, and structural analysis.

Small peptides react with DNS-Cl forming DNS-peptides. Their fluorescence intensities are quite stable. In our laboratory, the DNS-Naga spot on the polyamide sheet can be stored in the dark for a year without any apparent change of its fluorescence intensity. Since the structures of the peptides are different, the stability of their fluorescence intensities are different too. DNS-Cl not only reacts with the n-terminal amino group of the peptides, it can also react with the possible existing free hydroxyl and amino groups on the peptide chain. Therfore, the concentration of DNS-Cl in the experiment should be carefully controlled. For quantitative fluorescence measurement, the smallest amount of DNS-peptides can be determined is 10 p mole on the polyamide sheet and 0.1 p mole in the solution.

The fluorescence intensity of 1 n mole of DNS-Leu-enkephalin and DNS-Naga in methanol solution was measured. From low to high temperature, measurements were made for every increment of 5°C. The results are shown in figure 6. It can be seen that temperature does effect the fluorescence intensities of DNS-peptides. The temperature coefficient of DNS-Leu-enkephalin is 2 between 10°-20°C, i.e. for every 1°C increment of temperature, the fluorescence intensify decreases by only 0.5 percent. The temperature coefficient of both DNS-Naga and DNS-Leu-enkephalin are very small between 30°-40°C. The fluorescence intensity remains basically the same as temperature increases. The decrease of fluorescence intensity following theincrease of temperature is mainly due to the energy conversion within the molecules, i.e. the transition of the excitation energy to the potential energy, or losing energy to the solvent through collisions.



The effect of temperature on the fluorescence intensities of DNS-peptides
o- - -o DNS-Leu-enkephalin
•——• DNS-Naga
(a) Fluorescence intensity
(b) Temperature (°C)

Figure 6.

The DNS-Leu-enkephalin and DNS-Naga were separately dissolved in methanol, chloroform and acetone to make 1 n mole solutions. The fluorescence intensities of these solutions were then determined. Results are shown in table 3. It can be seen that acetone and chloroform cause quenching of the fluorescence. This may associate with the nature of the DNS-peptides in the solvent and the inward diffusion of the solvent molecules. Therefore, it is necessary to choose the proper solvents so that the sensitivity of the experiment can be higher. Besides, the fluorescence intensity is also influenced by pH. Fluorescence is stronger between pH 3-4.

Table 3. The effect of different solvents on the fluorescence intensity of DNS-peptides

Sample	Concentration	Fluorescence intensity (%)		
		Methanol	Chloroform	Acetone
DNS-LEK*	1	100	12.3	43.0
DNS-Naga	1	100	75.4	10.8

*Leu-enkenphalin

In this paper, the concentration of the DNS-peptides for the standard curves of quantitative fluorescence measurement are 10^{-13} -10^{-11} mole in solution and 10^{-11} - 10^{-9} mole on the polyamide sheets. The sensitivities for the fluorescence measurements of DNS-peptides in the solution or on the polyamide sheets are quite high. If the DNS-peptides used are beyond this concentration range, the instrument has to be adjusted so that higher resolution can be obtained. New standard curves have to be made after an adjustment.

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ISOLATION, PURIFICATION OF α_2-MACROGLOBULIN FROM HUMAN BLOOD PLASMA

Beijing SHENGWUHUAXUE YU SHENGWUWULI JINZHAN [BIOCHEMISTRY AND BIOPHYSICS] in Chinese No 3, Jun 86 pp 69-72

[Article by Peng Qiming [1756 0796 2494], China Medical Institute, Shenyang, and Zhang Shuqin [1728 3219 3830], Shenyang Mecical College; paper received 6 September 1985]

[Text] α_2-Macroglobulin (α_2M) is a high molecular weight plasma glycoprotein containing 8-11 percent carbohydrate. It has a molecular weight of 725,000 and an isoelectric point of 5.5. α_2M contains 4.8 gram atoms of zinc per mole, amounts to 20 percent of the total zinc in the serum.

α_2M is an important serum proteinases inhibitor. The concentration of α_2M in human plasma is about 2mg/ml. It can inhibit the activities of four different groups of endopeptidases. The nature of the molecular interaction between circulating proteolytic enzymes and their plasma inhibitors is not fully understood. When α_2M reacts with trypsin, thrombin, plasmin and kallikrein, a polypeptide (MW: 8500) was released from the α_2M molecule. The proteolytic attacks on the α_2M molecule result in a confromational change that the enzyme molecule is irreversibly trapped within the α_2M molecule. Access of substrates to the active site of the enzyme becomes sterically hindered, causing inhibition that is most pronounced with large substrate molecules but not small ones. This is the difference between α_2M and other proteinase inhibitors (1).

The main physiological function of α_2M is shown by its ability to inactivate proteinase. Once α_2M forms a complex with proteinase, the α_2M-proteinase complex is rapidly removed from the circulation. Therefore, α_2M may thus help in the regulation of the extracellular proteolytic activities. For example, proteinases released from granulocytes and other cells in inflammation can form complexes with α_2M molecules. These complexes are rapidly cleared by the reticulo-endothelial cells. However, under normal physiological conditions, the molecule of α_2M is too large to escape from the circulation in appreciable amounts. During inflammation, however, increased vascular permeability allows it to escape into the tissues. In many pathological states, α_2M accumulates in the extracellular fluid free or complexed with proteinases. For example, in pleuritis, peritonitis and arthritis, the complexes of α_2M-elastase and M-collagenase accumulate in the pleural fluid, ascites fluids

and synovial fluid, thus protecting the tissues from further degradation by the proteinases. Due to its broad actions, α_2M may participate in clotting, thrombo formation, bradykinin release and many important physiological and pathological processes. In order to study these functions, Schultz etal first isolated α_2M from human serum in 1955 (2). Since then, many articles have been published abroad on the various methods used for the separation and purification of α_2M, including precipitation, gel filtration, immunoadsorption, electrophoresis, etc. More recently, various chromatograph methods, such as cibacron blue sepharose chromatograph, have been used to purify α_2M (3-6). In this country however, there have been no reports published in this area.

Materials and Methods

Zn-chelated sepharose 6 B was a gift from professor Wang Tadong, National Institute of Health, U.S.A. Blue sepharose CL6B, trypsin and soybean trypsin inhibitor were obtained from Sigma. Benzoyl-DL-arginine-p-nitroanilide HCL (BAPNA) was supplied by Merck.

Measurement of α_2M Activity

The procedures were carried out according to the method of Ganrot with minor modifications. To 0.5 ml of sample materials was added 0.85 ml of Tris-HCL buffer, pH 8.2 containing 0.02 M $CaCl_2$ and 0.05 ml (2mg/ml) trypsin. The mixture reacted at room temperature for 3 minutes to form a trypsin-protein esterase complex. Soybean trypsin inhibitor at a volume of 0.05 ml (2mg/ml) was added to the complex mixture to inhibit the activity of excessive trypsin. Ten ml of BAPNA (0.003 M) solution was then added, after which the sample was incubated at room temperature for 10 minutes. The reaction was stopped after 10 minutes by adding 30 percent acetic acid. Since the trypsin-α_2M complex is still accessible to substrates of low molecular weight such as BAPNA, the hydrolytic activity of this complex on BAPNA produced a yellow product. A trypsin standard and a blank without trypsin but otherwise identical were incubated simultaneously. The activity of the α_2M trypsin complex is expressed by the absorbance of the sample at 410 mμ on a model 721 colorimeter.

Measurement of Protein Content Using the Folin-phenol Method

Methods for the separation of α_2M:

Step one: Polyethylene glycol (PEG) precipitation

Blood samples were obtained from healthy adult blood donors. To each 100 ml whole blood sample, 10 ml of 0.13 M sodium citrate, 10 mg soybean trypsin inhibitor and 40 mg polybrene were added. Plasma was separated by centrifugation. At 4°C, two volume of 0.02M NaH_2PO_4, 0.1 M NaCl, pH 7.4 were added to 18 ml of the plasma supernant. A 50 percent PEG was then slowly added to the mixture to achieve a 12 percent concentration. After mixing the centrifugation, the precipitate was harvested and contained the α_2M.

Step two: Zn-chelate sepharose 6B chromatograph separation

Zn-chelate sepharose 6B equilibrated with 0.02M NaH_2PO_4, and 0.5 M NaCl, pH 6.4 PEG plasma precipitate was dissolved in 7.5 ml of the above buffer and applied to the column. The first effluent peak was eluted with the same buffer. The second effluent peak was eluted by using 0.1 M sodium EDTA pH 7.0. The activity of α_2M was found in the second peak as shown in figure 1. The α_2M rich rubes No. 28-30 with a total volume of 10 ml were collected and subjected for the following steps.

Step three: blue sepharose CL6B chromatograph column (12.5x1.5cm) with a total volume of 22 ml was equilibrated with 0.50 M Tris-HCl buffer, pH 8.0. The 10 ml sample obtained from step 2 was adjusted to pH 8.0 by using 0.05 M Tris-HCl, pH 9.0. The first effluent peak was eluted by using 0.05 M Tris-HCl, pH 8.6. By increasing the ionic concentration, the second effluent peak was eluted with 0.05 M Tris-HCl, 0.5 M NaCl, pH 8.0. The α_2M activity was recovered in the first half of the first effluent peak as shown in figure 2. Tubes 3-4 containing α_2M rich elutions with a total volume of 10 ml were pooled for the following step.

Step four: Zn chelate sepharose 6B rechromatograph

After adjusting the 10 ml sample collected from step 3 to pH 6.4 with 2 M NaH_2PO_4 pH 5.5, the procedures of step 2 were followed. The protein content of the first effluent peak was relatively low. The α_2M activity was recovered in the second effluent peak as shown in figure 3. The 4 ml sample collected from tube No. 15 had the highest specific activity and contained the purified α_2M.

The four steps separation scheme described here have given a 72-fold purification of α_2M with a 31 percent recovery.

Determination of the Purity of α_2M

The purity of α_2M was determined by using 5-18 percent SDS Acrylamide gel slab electrophoresis. After running for 4 hours under a current of 40 mA, the gel was stained and rinsed. It can be seen from the electrophoresis pattern that the α_2M content in the plasma sample gradually increases after passing through each step of the four steps purification scheme. (the α_2M content in the plasma is extremely low, the band is very narrow and does not show in the photograph.) Protein contaminates gradually decrease. Finally, the purified product of α_2M forms a single band as shown by the electrophoresis pattern in figure 4.

Results and Discussion

α_2M is the inhibitor of all endopeptidases. Therefore, α_2M can easily react with plasma proteinases and lose its activities. α_2M can also combine with ammonium salt and becomes inactivated. During the past, $(NH_4)_2SO_4$ precipitation was employed to separate α_2M. Since this method may impair the molecule's

activity, it was abandoned. In this paper, we used polyethylene glycol stepwise precipitation to collect a concentration of 4-12 percent precipitation rich in α_2M with a 90 percent recovery of the activity. In order to avoid direct contact with a glass surface, all purification procedures were performed using plastic or siliconized glass wares which may prevent the glass-induced activation of Hageman factor-dependent pathways. Reactions were carried out in a 4°C cold room. Therefore, the specific activity of the final product was higher.

In order to separate α_2M with other macroglobulins in the plasma such as β-lipoprotein, fibrinogen, immunoglobulins M and A and heptoglobulin etc., we used a three-step chromatograph procedures. Zn-chelated sepharose is a relatively new metal-chelated chromatograph technique. Since α_2M is rich in sialic acid, it can combine with Zn ions to form a complex and adsorb unto sepharose. Therefore α_2M can be separate from other proteinases that have less a finity to the sepharose column. Blue sepharose CL6B contains pigment. It has a higher affinity for all enzymes using adenylic acid as co-enzymes than for α_2M. Blue sepharose CL6B also has high affinities with γ-globulin, different clotting factors and hemoglobulin. Therefore, α_2M can be separated from many other protein contaminates in the plasma. During separation, α_2M was the first fraction eluted from the column and formed a peak. α_2M activity was recovered from the first half of the peak.

Our purified α_2M formed a single band on the SDS-polyacrylamide gel electrophoresis. This result agreed with other electrophoresis patterns reported in literatures abroad. It proves that α_2M so obtained was higher purity and can be used for the preparation of antiserum.

We are grateful to professor Wang Tadong of the National Institute of Health, U.S.A. for his help in carrying out this work.

Table 1. Protein content and yield of α_2M in human plasma during the processes of separation and purification

Separation procedure	Volume ml	Protein concentration mg/ml	Activity mg/ml	Relative activity mg/mg	Total activity mg	yield %	Purification (fold)
Plasma	18.0	67.87	0.248	0.0037	4.46	100	1
First step	7.5	64.18	0.538	0.0084	4.04	90.7	2.3
Second step	10.0	10.54	0.270	0.0256	2.70	60.8	6.9
Third step	10.0	2.36	0.233	0.0986	2.34	52.0	26.7
Fourth step	4.0	1.30	0.347	0.2600	1.39	31.1	72.2

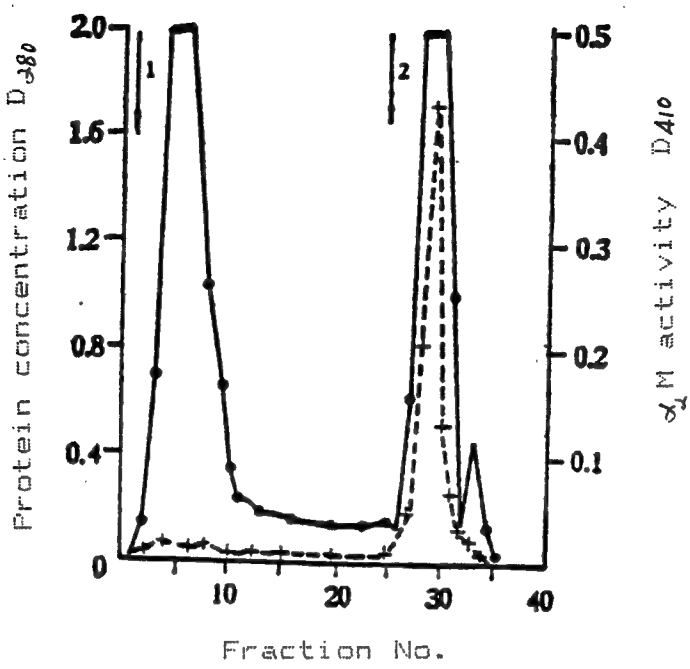

Figure 1. Elution profile of α_2M containing fractions by chromatograph of human plasma on Zn-chelated sepharose 6B Protein concentration —•—•— α_2M activity —+—+—
1: 0.02 M NaH_2PO_4, 0.5 M NACl, pH 6.4 buffer
2: 0.1 M Na_2EDTA, pH 7.0 buffer

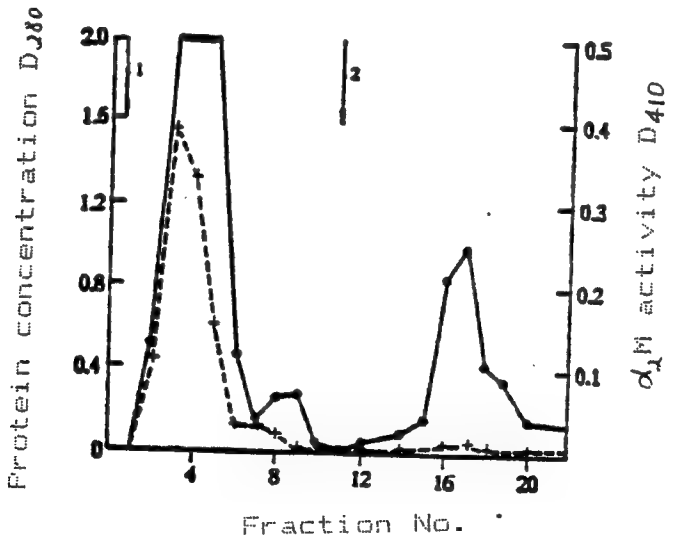

Figure 2. Elution profile of α_2M containing fractions by chromatograph of human plasma on blue sepharose CL6B Protein concentration —•—•— α_2M activity --+--+--
1: 0.05 M Tris-HCl, pH 8.0 buffer
2: 0.05 M Tris-HCl, 0.5 M NaCl, pH 8.0 buffer

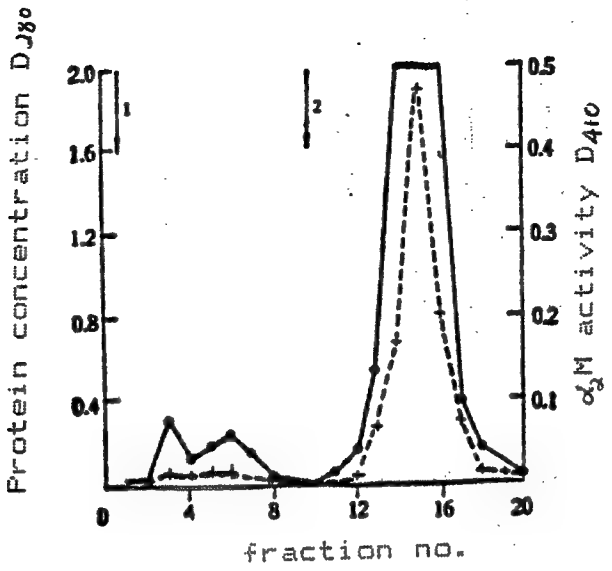

Figure 3. Elution profile of α_2M containing fraction by chromatograph of human plasma on Zn-chelate sepharose 6B for the second time.
Protein concentration _,—,—
$_2$M activity --+--+--
1: 0.02 M NaH_2PO, 0.5 M NaCl, pH 6.4 buffer
2: 0.1 M Na_2EDTA, pH 7.0 buffer

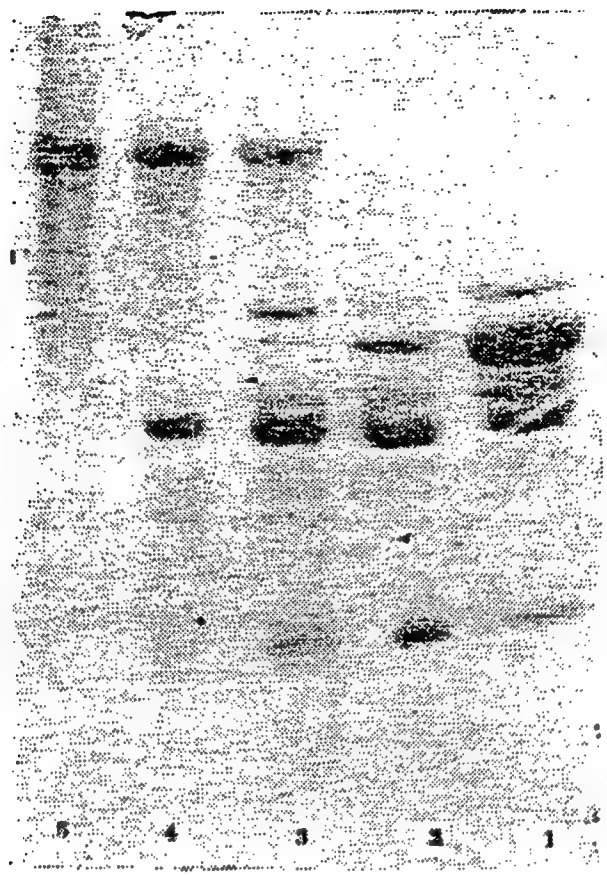

Figure 4. SDS-polyacrylamide gel electrophoresis
(Top: cathode; bottom: anode)
1: Plasma
2: 4-12 percent PEG precipitate
3: First chromatograph on Zn-chelate sepharose 6 B
4: Chromatograph on blue sepharose CL6B
5: Second chromatograph on Zn-chelate sepharose 6 B

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ASSAY FOR TRANSCRIPTION OF RAT AFP AND ALB GENES WITH IMMOBILIZED PROBE DNA DISC MEMBRANE

Beijing SHENGWUHUAXUE YU SHENGWUWULI JINZHAN [BIOCHEMISTRY AND BIOPHYSICS] in Chinese No 3, Jun 86 pp 46-48

[Article by Li Baogui, Wu Daqing, Zhong Jinliang, Huang Daopei, Ceng Qingyi, Chinese Academy of Sciences, Biochemistry Institute, Shenghai, and Lu Huiqing, Shanghai Bioproducts Research Institute; paper received 28 September 1985]

[Text] Fetoprotein (AFP) and albumin (Alb) are two major proteins with similar molecular weights. The amino acid sequence homologies between these two proteins and their similarity in secondary protein folding structure indicate that there is a close relationship between AFP and Alb in the evolutionary process. It is generally regarded that these two proteins are derived by duplication of a common ancestral gene and constitute a gene family. However, the developmental expressions of AFP and Alb genes are different. During the normal development of mammalian fetus, AFP is first synthesized by the yolk sac and later by the embryonic liver. It is the major serum protein in the developing fetus. During early postnatal life, serum level of AFP drastically decrease to a very low concentration. An extremely small amount of AFP is synthesized by the liver cells in adult life. The Alb serum concentrations follow an opposite pattern. They increase from low levels early in fetal development to high, approximately constant levels after birth. However, the phenotypic expression of AFP changes in hepatoma bearing animals. These animals have highly elevated levels of serum AFP. These changes have been used clinically as an indicator for the early detection of malignant transformation of liver cells. The changes in serum concentrations of AFP and Alb are regulated by the concentrations of the corresponding m-RNA. A number of laboratories have used AFP and Alb as indicators for their studies on the mechanisms of hapatocarcinoma transformation and the eukaryotic gene regulation. Therefore, detecting the changes of AFP and Alb m-RNA level have provided insight in the understanding of the mechanisms that control the gene expression. This paper introduces a simple method of using immobilized probe CNA disc membrane to qualitatively and quantitatively measure the AFP and Alb gene transcription products--m-RNA AFP and m-RNA Alb. The special property of the DISC method is that different plasmid DNA probes can be fixed on the same membrane and then hybridized with radioactive RNA. In this paper, plasmid DNA containing rat AFP and Alb gene fragments were fixed on cellulose nitrate paper and hybridized with ^{125}I labeled rat poly A+ RNA. The alterations in the expression of these two genes are indicated by their radioautographs and the amount of radioactivities.

Materials and Methods

Rat embryonic liver and yolk sac were obtained from 16-18 day old rat embryos. Normal rat livers were obtained from healthy adult male rat. Agarose (Shanghai Donghai pharmaceutical Co.). Guanidinium thiocynate (Shanghai Second Testing Reagent Factory). Cellulose nitrate filters (S&S BA 85 0.45 μm). Yeast t-RNA (Shanghai Dongfeng Bio-reagent Co.). Restriction endonucleases Pst I (BRL). Ficall 400 Pharmacia). Polyacrylamide (PVP) (Sigma). Rat AFP (pRAF 87, pRAF 65) (pRSA 13, pRSA 57) and albumin were supplied by Dr. Sargent, U.S.A.

1. Purification and labelling of Poly A+ RNA extracted from different tissues of rats.

Total RNA were prepared from fresh or liquid nitrogen preserved rat liver, 16-18 day old embryonic rat liver and yolk sac according to the guanidinium thiocynate method by Chirgwin (9) (omitting the step of cesium chloride ultracentrifugation.) Total poly A+ RNA were prepared from total RNA through two cycles of binding to oligo (dt)- cellulose labeled with ^{125}I according to the method described before (10). The volume was reduced to 150 μl, and the samples were shaked for 102 minutes at a constant temperature.

2. Electrophoresis of RNA

The method described by Liu Duohua was essentially followed. The concentration of agarose was 1 percent. Each sample contained 10 μg RNA.

According to the method of Dagert, plasmid DNA of PRAF 87, pRAF 65, pRSA 13 and pRSA 57 containing gene setments of AFP and Alb were added to CaCl treated E. coli 600 cell suspension and then proceeded with increasing in DNA concentration and purification (10). Purified plasmid DNA was identified by the restriction map of PstI restriction enzymes and Southern hybridization method.

4. Preparation of DNA Disc membrane and quantitative and qualitative hybridization measurement of m-RNA AFP and m-RNA Alb.

Equal amounts of pRAF 87 and pRAF 65 containing rat AFP genes and pRSA 13 and pRSA 57 containing rat Alb genes were mixed in 4x SSC solution. The final concentration was 0.2 μg/ul. According to the previously described method, 5μl samples were loaded on the pre-treated cellulose nitrate filter membrane. An equal amount of pBR 322 was used as a standard for making the DISC membrane. DNA on the DISC membrane was hybridized with ^{125}I labeled rat poly A+ RNA. Results were expressed by the radioautographs of the hybrides and the amount of radioactivity. The former represents the qualitative results, the later the quantitative results.

Results and Discussion

1. Quality comparison of poly A+ RNA and radioactive labeling.

During the processes of poly A+ RNA preparation, caution must be taken to prevent the hydrolysis of RNA by RNAase. In this experiment, besides rendering all glasswares nuclease free by sterilization, tissues were homogenized with full speed in a strong denaturant, guanidinium thiocynate in order to reduce the hydrolysis of RNA by RNAase. This was the first step for the extraction of poly A+ RNA as described by Chirgwin. Poly A+ RNA were separated from crude total RNA by using Oligo (dT) - cellulose (odc). This method is not only simpler, the poly A+ RNA so obtained also have larger molecular weight and higher yields in comparison with those obtained from polysomes prepared from tissue homogenates. (picture 1). Since oligo (dT)- cellulose column was used to purify m-RNA, contamination by r-RNA could occur. However, the data indicates that after two cycles of binding to oligo(dT)-cellulose, m-RNA so obtained can be used directly for radioactive labeling. Since the volume of labeled poly A+ RNA was reduced and the reaction system was under strict control so that the pH is under 5, the specific activity of ^{125}I labeled poly A+ RNA was higher. Generally it can reach to $4\text{-}6\times10^{7}$ cpm/μg. Since ^{125}I is cheaper, has a longer half life and does not need to be kept cold during transportation, it can be used to replace α^{32}-PdNTP. Therefore, ^{125}I is mor suitable for our country's need, economically and time wise.

2. Identification of hybrides between rat total poly A+ RNA extracted from different tissues and rat AFP and Alb probes.

Pst I restriction enzyme digested and undigested plasmid DNA containing AFP and Alb gene sequences were transferred to cellulose nitrate filter membrane and then hybridized with ^{125}I labeled rat total poly A+ RNA. Results show that poly A+ RNA from normal rat liver can only hybridize with plasmid DNA containing Alb gene sequence and Alb gene segments. Poly A+ RNA from yolk sac can only hybridize with plasmid DNA containing AFP gene sequence and AFP gene segments. On the other hand, Poly A+ RNA from rate embryo liver can hybridize with both. (picture 2, 3) These results are essentially the same as those reported previously (1, 2). It proves that purified DNA probes and poly A+ RNA extracted from different rat tissues can be used for hybridization studies.

3. Qualitative and quantitative measurements of rat m-RNA AFP and m-RNA Alb obtained from different tissues.

Three of the same kind of DISC membranes containing plasmid DNA of pBR 322, pRAF and pRSA were hybridized under the same condition with ^{125}I labeled total poly A+ RNA extracted from different rat tissues. After hybridization, the presence or absence of m-RNA AFP or m-RNA Alb in the tissues can be determined by the radioautographs of the hybrides on the DISC membranes. Preliminary estimation can also be made on the relative quantities of the two m-RNA. Results so obtained agree with those obtained by using the Southern hybridization method. The relative quantities of m-RNA AFP and m-RNA Alb and their ratio can be determined by measuring the radioactivity of the probe spots on the DISC membrane (table 1).

Table 1. Relative quantities of m-RNA AFP and m-RNA Alb obtained from different tissues of rat

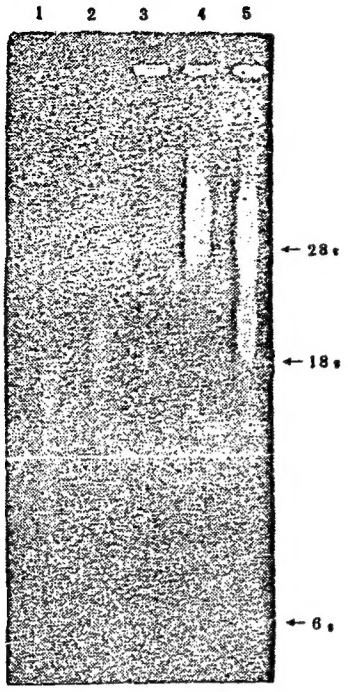
Tissue	DISC (cpm) mRNA AFP	mRNA Alb	mRNA AFP/mRNA Alb
Normal adult rat liver	11	1577	0.007
Rat embryo liver (16-18 days)	561	840	0.68
Rat yolk sac (16-18 days)	1139	1	1139.0

*Substracted the background counts of pBR322

The results above agree with those obtained by using other methods as reported in literature (15-17). This shows that the method used here can be employed for the qualitative and relative quantitative determination of rat mRNA AFP and m-RNA Alb. Therefore, this procedure is a convenient method to measure quantitatively the alterations of the expression of AFP and Alb genes. If competitive hybridization method is used, either poly A+ RNA containing m-RNA AFP form rat yolk sac, or poly A+ RNA containing m-RNA Alb from rat liver needs to be labeled. Poly A+ RNA extracted from different experimental materials can be added to the labeled m-RNA during hybridization. From the level of competitative inhibition, the quantitative levels of the two gene transcription products of the experimental materials can then be determined. More results can be obtained in one single experiment by increasing the number of sample spots on the DISC membrane.

The quality of the DISC membrane can directly affect the results of the measurement. The DNA probes should be evently distributed on the DISC membrane. Otherwise, results can also be affected. High purity and good solubility of DNA molecules are the keys for the preparation of an even DISC membrane.

We are grateful to professor Zhou Guangyu and professor Qiu Zhengfu of the University of Vermont, for their help in carrying out this work.



1. Normal rat liver poly A+ RNA purified by one cycle of binding to odc
2. Normal rat liver poly A+ RNA purified by two cycles of binding to odc

Guanidinium thiocyanate extraction and two cycles of binding to odc:

3. Rat yolk sac poly A+ RNA
4. Rat embryonic liver poly A+ RNA
5. Rat liver poly A+ RNA

Plate 1.
Elec-rophoresis of poly A+ RNA obtained by using different methods:

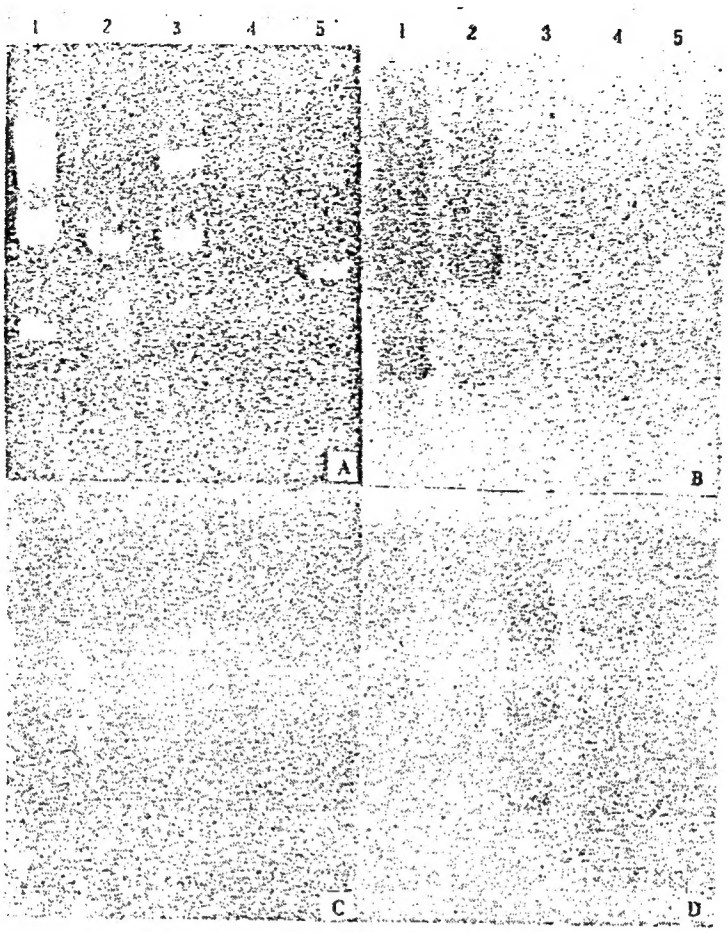


Plate 2.

Electrophoresis and hybridization of rat AFP and ALB plasmid DNA

A. Electrophoresis of plasmid DNA
B. Hybridization of A transfer member and ^{125}I labeled normal adult rat liver poly A+ RNA
C. Hybridization of A transfer membrane and ^{125}I labeled rat embryonic liver poly A+ RNA
D. Hybridization of A transfer membrane and ^{125}I labeled rat yolk sac poly A+ RNA
    1. pRSA 13
    2. pRSA 57
    3. pRSA 65
    4. pRAF 87
    5. pBR 322

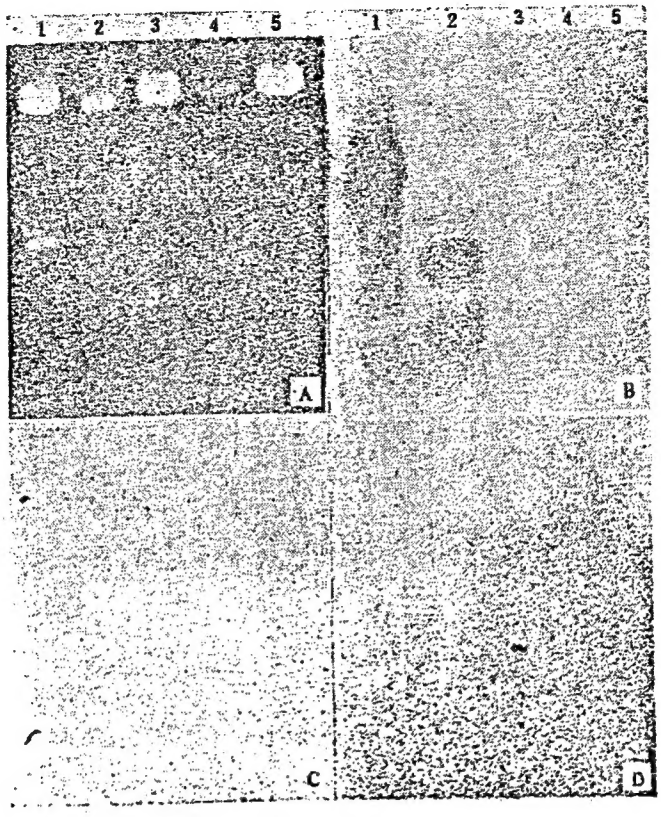


Plate 3.

Hybridization and electrophoresis of Pst I digested AFP and Alb plasmid DNA

A. Pst I digested plasmid DNA analyzed by electrophoresis
B. Hybridization of A transfer DISC membrane and ^{125}I labeled normal adult rat liver poly A+ RNA
C. Hybridization of A transfer DISC membrane and ^{125}I labeled rat embryonic liver poly A+ RNA
D. Hybridization of A transfer DISC membrane and ^{125}I labeled yolk sac poly A+ RNA
    1. pRSA 14 + Pst I
    2. pRSA 57 + Pst I
    3. pRAF 65 + Pst I
    4. pRAF 87 + Pst I
    5. pBR 322 + Pst I

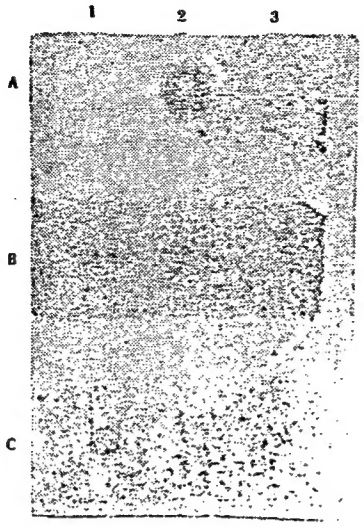


Plate 4.

Hybridization of Probe DISC membrane and poly A+ RNA obtained from different tissues

A. Normal adult rat liver
B. Rat embryonic liver
C. Rat yolk sac
   1. pRAF 65 + pRSA 87 AFP DISC spot
   2. pRSA 13 + pRSA 57 Alb DISC spot
   3. pBR 322 reference spot

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